AUG 15 1938

86th Annual Meeting of the American Pharmaceutical Association and Related Organizations, Minneapolis, Minnesota, August 21st to 27th. Headquarters, Hotel Nicollet. See Your Transportation Agent for Rates and Routes.

Vol. XXVII, No. 7 JULY, 1938

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

TRADE MARK

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Published Monthly by the American Pharmaceutical Association: Office of Publication, 20th & Northampton Sts., Easton, Pa. Editorial Office, 2215 Constitution Ave., Washington, D. C. Annual Subscription, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, 35 cents; Canada, 40 cents; Other Foreign, 50 cents.

THE 86th ANNUAL MEETING OF THE AMERICAN PHARMACEUTICAL ASSOCIATION WILL BE HELD IN MINNEAPOLIS, MINN., Hotel Nicollet, August 22–27, 1938.

The 1938 Meetings of the American Association of Colleges of Pharmacy, the National Association of Boards of Pharmacy, Conference of Pharmaceutical Association Secretaries, of the Pharmaceutical Law Enforcement Officials and the Plant Science Seminar will also be held in Minneapolis, Minn.

Entered as second-class Matter, January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879. Acceptance for mailing at a special rate of postage provided for in section 1103. Act of October 3, 1917; authorized July 10, 1918.

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GENERAL PROGRAM FOR THE EIGHTY-SIXTH ANNUAL MEETING OF THE AMERICAN PHARMACEUTICAL ASSOCIATION AND RELATED ORGANIZATIONS, NICOLLET HOTEL, MINNEAPOLIS, MINNESOTA, AUGUST 21–27, 1938.

AUGUST 15TH–19TH.

Plant Science Seminar.

FRIDAY, AUGUST 19TH.

The American Council on Pharmaceutical Education and the A. PH. A. Committee on National Formulary will hold Sessions at 9:30 A.M. and 2:30 P.M., on Friday and Saturday, August 19th and 20th, respectively.

SATURDAY, AUGUST 20TH.

2:30 P.M. National Conference Pharmaceutical Research.
6:30 P.M. Dinner, N. C. P. R.
8:30 P.M. Evening Meeting, N. C. P. R.

SUNDAY, AUGUST 21ST.

9:30 A.M. Council, A. PH. A.
2:00 P.M. Meeting, Committee on Pharmaceutical Syllabus.
9:00 P.M. Opening Concert.

MONDAY, AUGUST 22ND.

9:00 A.M. A. A. C. P.—Executive Committee.
9:30 A.M. Chemistry Conference.
Pharmacy Conference.
Pharmacognosy and Pharmacology Conference.
Pharmaceutical Economics Conference.
9:30 A.M. N. A. B. P.
1:00 P.M. Luncheon and Style Show (Ladies).
1:30 P.M. A. A. C. P.
1:30 P.M. N. A. B. P.
6:30 P.M. Joint Dinner, N. A. B. P. and A. A. C. P.
8:00 P.M. A. A. C. P.
8:30 P.M. First Session, Conference Pharm. Law Enforcement Officials.

TUESDAY, AUGUST 23RD.

9:00 A.M. Sightseeing Trip to St. Croix Falls, returning via River Road, Luncheon (Ladies).
9:30 A.M. Joint Conference, A. PH. A., N. A. B. P. and A. A. C. P.
2:00 P.M. N. A. B. P.
2:00 P.M. A. A. C. P.
6:30 P.M. Dinner, Rho Chi Fraternity, followed by Annual Meeting.
8:00 P.M. First General Session, A. PH. A.
10:30 P.M. Smörgåsbord and Entertainment.

WEDNESDAY, AUGUST 24TH.

8:00 A.M. Breakfast, Members U. S. P. Committee of Revision and Board of Trustees.
9:30 A.M. First Session, House of Delegates, A. PH. A.
12:30 P.M. Luncheon (Ladies), followed by a meeting of the Women's Auxiliary of the A. PH. A.
12:45 P.M. Luncheon, Kappa Psi Fraternity and Lamba Kappa Sigma Sorority.
12:45 P.M. Luncheon, Phi Delta Chi Fraternity.
2:30 P.M. Council, A. PH. A.
2:30 P.M. First Session, Scientific Section.
2:30 P.M. First Session, Section on Education and Legislation.
2:30 P.M. First Session, Section on Practical Pharmacy and Dispensing.
2:30 P.M. First Session, Sub-Section on Hospital Pharmacy.
2:30 P.M. First Session, Section on Historical Pharmacy.
2:30 P.M. First Session, Section on Pharmaceutical Economics.
2:30 P.M. First Session, Conf. Pharm. Assn. Secretaries.
3:30 P.M. First Session, Sub-Section on Pharmacognosy.
3:30 P.M. Conference of State Committees on U. S. P and N. F. promotion.
3:30 P.M. Meeting, Committee on Nominations.
3:30 P.M. Meeting, Committee on Resolutions.
6:30 P.M. President's Reception.
7:30 P.M. Joint Banquet, A. PH. A. and Related Organizations.

THURSDAY, AUGUST 25TH.

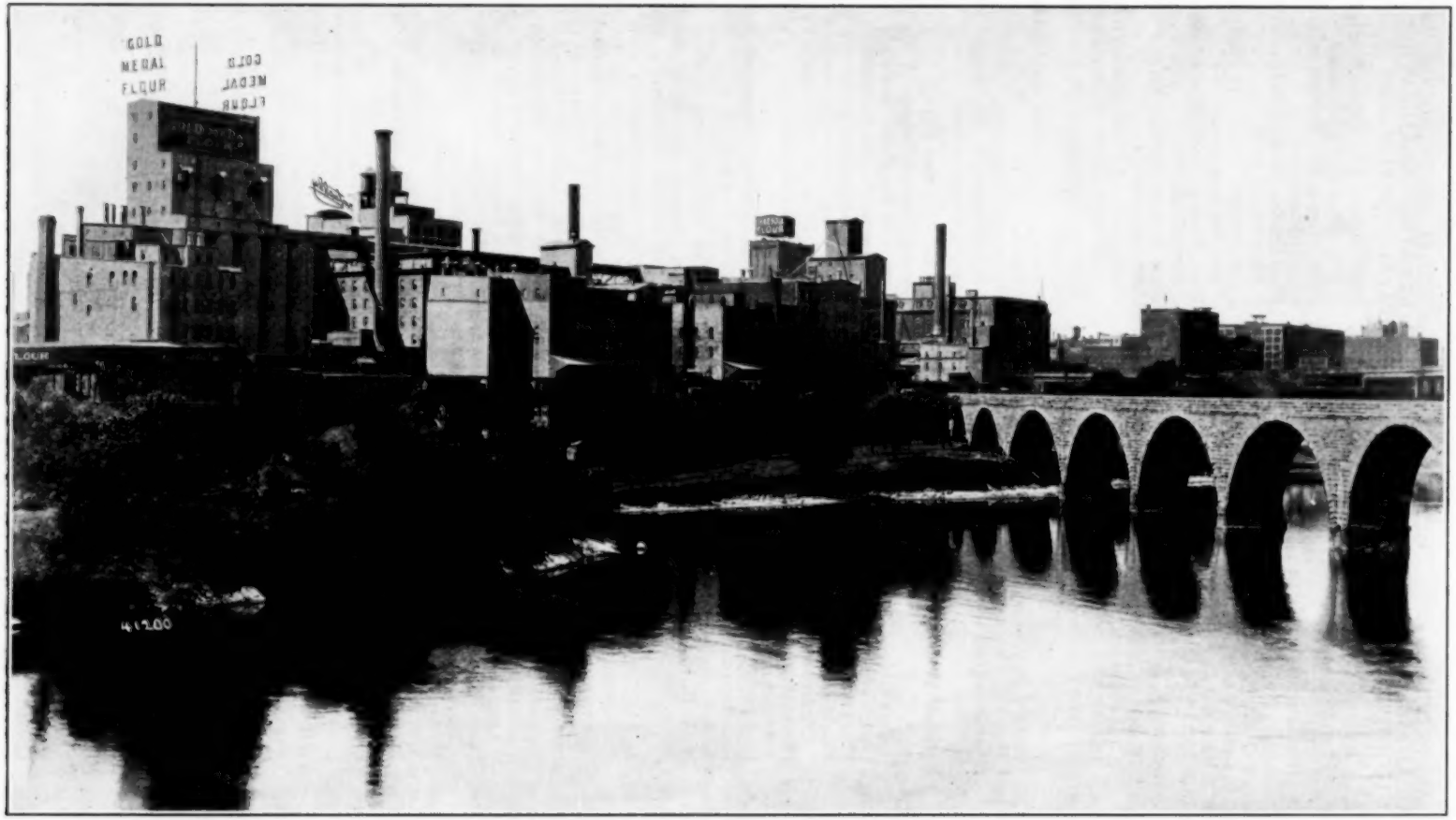
9:30 A.M. Second General Session, A. PH. A.
12:00 M. Luncheon for Honor Guests.
12:00 M. Luncheon—Kappa Epsilon.
12:15 P.M. Veteran Druggists' Luncheon.
1:30 P.M. Sightseeing Tour—University, College of Pharm., Minnehaha Falls, Lake Minnetonka, etc.
6:00 P.M. Dinner.
8:30 P.M. Second Session, House of Delegates, A. PH. A.

FRIDAY, AUGUST 26TH.

9:30 A.M. Joint Session, Scientific Section and Section on Practical Pharmacy and Dispensing.
9:30 A.M. Joint Session, Section on Education and Legislation, Conf. Law Enforcement Officials and Conf. Pharm. Assn. Secretaries.
10:30 A.M. Second Session, Sub-Section on Hospital Pharmacy.
10:30 A.M. Second Session, Scientific Section.
10:30 A.M. Breakfast (Ladies).
12:15 P.M. Special Luncheons.
2:30 P.M. Card Party (Ladies).
2:30 P.M. Third Session, Scientific Section.
2:30 P.M. Second Session, Section on Education and Legislation.
2:30 P.M. Second Session, Section on Practical Pharmacy and Dispensing.
2:30 P.M. Second Session, Section on Historical Pharmacy.
2:30 P.M. Second Session, Section on Pharmaceutical Economics.
2:30 P.M. Second Session, Conf. Law Enforcement Officials.
2:30 P.M. Second Session, Conf. Pharm. Assn. Secretaries.
6:30 P.M. Dinner, Former Presidents, A. PH. A.
7:30 P.M. Meeting, Committee on Resolutions.
8:00 P.M. Final Session, House of Delegates, A. PH. A.
10:30 P.M. Farewell Dancing Party.

SATURDAY, AUGUST 27TH.

9:30 A.M. Final General Session, A. PH. A.
1:00 P.M. Council, A. PH. A.



A BREAD AND BUTTER SKYLINE.

The Internationally Famous Skyline of the "Flour City." The late Joseph Pennell said, "I have found it (the character of Minneapolis) down by the falls of the river and it is the character, the grandeur and the beauty of usefulness. It is in the mills, these mills were not built to be imposing, impressive. They are impressive from their needs, their site, their form, their skyline and the way they have grown above the rushing river, the way they are shrouded in the mists of the morning, the way they loom and grow into a mighty mass when the sun sets behind them."

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOL. XXVII JULY, 1938 No. 7

A MESSAGE FROM THE PRESIDENT.

The arrangements for the Minneapolis meeting are now practically completed. The General Program given on a preceding page indicates that the attendants will have a wide choice of professional sessions interspersed with enjoyable entertainment features. Local Secretary Rogers and the pharmacists of the city and state having done all that they could in advance for the comfort and pleasure of their guests, are ready to welcome them.

As President, it is my pleasant duty to cordially invite the pharmacists of America to attend the Eighty-Sixth annual meeting of their ASSOCIATION and to assure them a cordial reception. We trust that they will bring their families and friends and that others interested in our profession will also be present. This should be one of the best attended and most successful meetings in our history.

The sessions of the A. PH. A. and of the organizations that meet with it cover every phase of professional pharmacy. The important professional problems are all considered in one division or another. Some of the present problems are pressing and deserve our best attention. A number of prominent speakers will discuss subjects of vital interest to Pharmacy, including food and drug legislation, the study of medical services, hospital pharmacy, the national health program and others.

Every pharmacist can find something of interest and value at this meeting and it affords a splendid opportunity to meet your fellow pharmacists and to learn what is being done elsewhere.

The underlying purpose of this meeting is to help the pharmacists themselves, to help them give a better service, and to acquaint those from elsewhere with the splendid pharmacists and other people of the Great Northwest.—E. N. GATHERCOAL.

The Internationally Famous Skyline of the "Flour City." The late Joseph Pennell said, "Have you seen the Falls (the Falls of St. Anthony) and the mills? Unsightly, are they? Not at all. They are beautiful. They are impressive from their needs, their site, their form, their skyline and the way they have grown above the rushing river, the way they are shrouded in the mists of the morning, the way they loom and grow into a mighty mass when the sun sets behind them."

EDITORIAL

E. G. EBERLE, EDITOR 2215 Constitution Ave., WASHINGTON, D. C.

IMPORTANT CONFERENCES.

THE AMERICAN PHARMACEUTICAL ASSOCIATION has been represented at two recent conferences, referred to below in separate articles, which may have an important bearing, particularly in one case, on the future of the profession. The Chairman of the Committee on the Study of Pharmacy, Dr. Robert P. Fischelis, represented the ASSOCIATION at these meetings. Other officers of the ASSOCIATION are also keeping in close touch and the members of the profession may rest assured that future developments in both movements will be carefully followed.

NATIONAL COUNCIL CONFERENCE.

ABOUT two years ago U. S. Senate Resolution No. 114, "to Investigate the Desirability of Establishing a National Economic Conference" was referred to the Committee on Manufactures, and has been under study since that time. Recently, an Advisory Committee for the proposed Council was appointed, the members of which represent the professions, commerce, sciences and arts.

In opening a meeting of the Advisory Committee with members of the Senate Committee on Manufactures, held in Washington on May 25, 1932, Senator Bulkley said: "Gentlemen, as you know, we have been working for more than two years on a plan for the establishment of what we started to call a 'National Economic Council,' and what we now like better to call a 'National Council,' because we realize that economics can hardly be separated from any other subjects that affect the welfare of the Nation. . . . . What we have in mind is a National Council made up of full-time members—perhaps of the number of nine, although no number has been finally and definitely decided upon, to give their entire time to the consideration of every problem that affects the National Government. The Council should be independent, and independent in the full sense, because if the President or the Congress can direct the Council to give its attention to any one thing, the directions may be so multiplied that the Council will not be able to give attention to the things that it thinks are most important. . . . . Now of course we propose that this council shall not have any administrative or executive authority, nor any authority to carry into effect its recommendations in any way."

The members of the Advisory Committee were invited to give their criticisms and suggestions, many of which were favorable and some unfavorable. The following quotations are from the statement made for this ASSOCIATION.

"The AMERICAN PHARMACEUTICAL ASSOCIATION feels, as many do here to-day, that this Council, if set up will be of great help in bringing to the members of the Federal and State Legislatures and to the Bureaus of the Government, the kind of impartial information which is needed as a basis for progressive legislation and administrative action. . . . . It recognizes the need of an impartial source of information as a substitute for propaganda and the acceptance of the views of pressure groups."

National Health Conference.

IN OCTOBER 1936, and following the passage of the Social Security Act, President Roosevelt issued Executive Order No. 7481, appointing an Interdepartment Committee to Coördinate Health and Welfare Activities with Assistant Secretary of the Treasury, Miss Josephine Roche, as the Chairman and stating that "It shall be the duty of this Committee (1) to continue to sponsor appropriate coöperative working agreements among the various agencies of the Government in the health and welfare field, and to continue the work under agreements already in effect, and (2) to study and make recommendations concerning specific aspects of the health and welfare activities of the Government looking toward a more nearly complete coördination of the activities of the Government in these fields. The Committee will continue to function through the special technical committees it has set up from time to time, and will designate additional committees to deal with new problems."

The Interdepartmental Committee appointed a Technical Committee on Medical Care, which after a general survey submitted a report on The Need for a National Health Program, including definite recommendations. This report was approved by the former committee and filed with the President on February 14, 1938.

The Interdepartmental Committee recently invited about two hundred delegates to consider a National Health Program at a National Health Conference held at the Mayflower Hotel, Washington, D. C., on July 18 to 20, 1938. For three days representatives of all branches of the Medical profession, including Pharmacy; Labor, Agriculture, Industry, Women's Organizations, and Health Organizations, Public Health Officials, Hospital Executives, Social Workers, Journalists and others participated in the Conference.

The report of the Technical Committee was made the basis of the Conference and the program was considered *first*, from the standpoint of national health needs; *secondly*, from the standpoint of expansion of general Public Health Services, such as expansion of Maternal and Child-Health Service; *thirdly*, from the standpoint of Hospital Facilities to provide medical care for the medically needy; and finally, from the standpoint of providing insurance against loss of wages during sickness.

There has been considerable discussion in the public press of the apparent need for and lack of medical care for people in all income groups and the indigent, based on various governmental and private surveys.

The Technical Committee reported that preventive health services for the nation as a whole are grossly insufficient; that hospital and other institutional facilities are inadequate in many places; that one-third of the population, including persons with or without income, is receiving inadequate or no medical care and that an even larger fraction of the population suffers from economic burdens created by illness.

To remedy these conditions five definite recommendations were proposed with approximately one-half of the estimated total annual cost of the first three to be met by the Federal Government. They are briefly stated as follows:

1. Expansion of Public Health and Maternal and Child-Health Services, involving by the tenth year, a total annual expenditure of $165,000,000.

2. Expansion of Hospital Facilities including construction and maintenance for general and specialized care and involving an average annual cost over a ten-year period of $146,050,000.

3. Grants-in-aid to the states from the Federal Government to implement the provision of public medical care (1) to those for whom local, state or Federal governments, jointly or singly, have already accepted some responsibility through the public assistance provisions of the Social Security Act, through work-relief programs, or through provisions of general relief; (2) to those who, though able to obtain food, shelter and clothing from their own resources, are unable to procure necessary medical care. It is estimated that $400,000,000 would eventually be required annually to provide minimum care to the medically needy.

4. A comprehensive program designed to increase and improve medical services for the entire population directed toward closing the gaps in a health program of national scope, left in the provisions of the first three recommendations. General taxation or special assessments alone, or in combination with specific insurance contributions from the potential beneficiaries of an insurance system, were recommended as the method of financing. The rôle of the Federal Government in this program is to be principally that of giving financial and technical aid to the states in their development of sound programs through procedures largely of their own choice.

5. Insurance against loss of wages during sickness.

The estimated annual cost of the program, without recommendations four and five, at the full level of operation within a ten-year period is $850,000,000.

The proposed program was received by members of the Conference with a variety of reactions. Social workers, health officers and others who are students of the health and welfare conditions of the people of the United States as a whole, reacted favorably. Some representatives of the medical profession and of private hospitals and institutions were inclined to question the necessity for embarking on a program of such great magnitude. Representatives of labor and agriculture and women's organizations strongly favored the Government proposals, and a substantial body of medical opinion likewise expressed a favorable reaction to extending medical services by some method other than that of private practice.

On the whole, those who whole-heartedly favored the proposals were in the majority. Those disposed to disapprove did not burn their bridges but contented themselves with questioning the emphasis placed upon the national need for medical care as compared with the need for food, fuel, clothing, shelter and jobs. Representatives of industry expressed themselves as not too greatly worried about the cost of the program as compared with the expected reduction in needless loss of life and suffering and increasing prospects of longer years of productive, self-supporting life.

No definite action was taken by the Conference on the proposals although it is expected by all participants that the groundwork for a national health program has been laid and that discussions from now on will center around the proposals advanced. That the revision of existing methods of providing medical care is actually under way has been apparent to anyone who has watched the recent venereal disease and pneumonia programs. The National Health Conference served as a means of crystallizing thought on immediate needs and future action.

As pharmacists we shall have an important part in whatever methods of action may be developed. The following quotations are taken from a statement made for the ASSOCIATION.

"The production and distribution of drugs and medicines constitute an important factor in any program of medical care. Thus far it has received little or no attention in the deliberations of this Conference, nor has it been specifically alluded to in the proposed National Health Program.

"The public's expenditure for drugs and medicines in 1929 was estimated at $715,000,000. It probably dropped considerably during the depression, but it is climbing again. It has been amply demonstrated that the business in drugs and medicines although affected to some degree by the incidence of illness, actually rises and falls with general business conditions. The implications in this statement seem worthy of consideration.

"The magnitude of the normal expenditure for drugs is not far below the estimated cost of the entire National Health Program submitted to this Conference. . . . . The limitation of the sale of drugs to pharmacies and pharmacists offers the best measure of control and reduction in public expense for drugs now available.

"The proposed programs for the control of venereal disease, pneumonia and other diseases have had the whole-hearted support of the pharmacists of the Nation, even though they have seen the distribution of vast amounts of drugs taken out of their hands and transferred directly to health departments.

"Pharmaceutical education and licensure is to-day on a high plane and the products of our colleges of pharmacy, although far fewer in number than ten years ago, are much better prepared to join in maintaining any acceptable national health program.

"The experience with Health Insurance in Great Britain has demonstrated that drug costs can be substantially lowered by limiting prescribing to official and approved drugs. The increase in available medical care and consequent increase in prescription writing has served to increase the professional activity of pharmacists and has reduced self-medication.

"It can be definitely stated that the pharmacists of the United States are well prepared to supply all necessary services in this field no matter what system or program of health care may emerge from this Conference."—E. F. K.

Dr. F. A. Gilfillan, who has been Professor of Pharmacy, in the School of Pharmacy, State College of Oregon, Corvallis, since 1927, has recently been appointed as Acting Dean of the School of Science of the College which comprises the Departments of Bacteriology, Botany, Chemistry, Entymology, General Science, Geology, Mathematics, Nursing Education, Zoölogy and premedical work. This school will have eighty persons on its staff.

After graduating from the School of Pharmacy in 1918, Dr. Gilfillan enlisted in the Chemical Warfare Service and was stationed at Yale University. Later, he completed the requirements for the M.S. and Ph.D. degrees in the University. From 1921 to 1925, he was a member of the staff of the School of Pharmacy, State College of Oregon and from 1925 to 1927, of the School of Pharmacy, University of Florida. He is a member of Sigma XI, Rho Chi, Phi Beta Phi, a number of several honorary scientific societies and of the AMERICAN PHARMACEUTICAL ASSOCIATION.

SCIENTIFIC SECTION

BOARD OF REVIEW OF PAPERS.—*Chairman*, F. E. Bibbins; H. M. Burlage, W. G. Crockett, E. V. Lynn, C. O. Lee, L. W. Rising, L. W. Rowe, Heber W. Youngken, Ralph E. Terry, Carl J. Klemme.

THE BIOASSAY OF DIGITALIS WITH OBSERVATIONS ON THE p_H FACTOR.*[1]

BY CASIMIR T. ICHNIOWSKI AND MARVIN R. THOMPSON.[2]

INTRODUCTION.

The literature is replete with reports showing that there is much disagreement as to the selection of the best method for the assay of Digitalis.

A problem concerning the assay methods for Digitalis in this laboratory centered itself about the cat or frog methods because most of the preparations of Digitalis which are placed at the disposal of the practicing physician are standardized by one or another of the several frog methods. A review of the literature likewise shows that the most important methods used in the standardization of Digitalis are based on procedures using the cat or the frog as the test object.

The following reports bear out the above statement: Hatcher and Brody (1), Haskell (2), Eckler (3), Eggleston (4), Rowntree and Macht (5), Colson (6), Rowe (7), O'Brien and Snyder (8), Haskell, Dowell and Terry (9), A. D. M. A. Proceedings (10), Haskell and Courtney (11), Haskell (12), Pittenger (13), Vanderhoff (14), Wible (15), Trevan, Boock, Burn and Gaddum (16), Rowe (17), Swanson and Hargreaves (18), Wokes (19), Edinburgh Conference of the League of Nations Health Committee (20), the Geneva Conference (21), Knaffl-Lenz (22) and the Frankfurt Conference (22).

It is significant that in the application of the cat method in the present work, ouabain was not used to terminate the tests, nor was the "Cat Unit" (based on ouabain) method of expressing potency peculiar to the Hatcher-Brody technique, employed in these studies. It is still more significant that the ouabain-complicating "Cat Unit" procedure of Hatcher and Brody apparently has been abandoned in Dr. Hatcher's own laboratory (23). The confusion is left remaining, however, because in spite of the fact that Digitalis alone is used in the procedure described, it is still referred to as the "Hatcher-Brody method" in the article (23).

The 1932 edition of the British Pharmacopœia requires that Digitalis and its preparations be standardized in terms of the International Standard Digitalis Powder and that in the actual assay, comparison of an unknown is made against the official standard preparation of powdered Digitalis. The frog method involving the use of a mortality curve, the procedures involving the use of the guinea pig or the cat slowly perfused with an infusion of the powder or a saline dilution of the tincture are listed by the B. P. as methods that are satisfactory.

The Heart Committee (23) of the N. Y. Tuberculosis and Health Association recommends that the Hatcher-Brody procedure be used in standardizing Digitalis,

* Presented before the Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] Abstracted from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] From the Department of Pharmacology, School of Pharmacy, University of Maryland.

but as pointed out in the preceding paragraphs, the procedure used is not that of Hatcher and Brody (1) since ouabain is not involved in the test.

Edmunds (24), in replying to this recommendation of the Heart Committee, stated that the Revision Committees of the U. S. P. voted unanimously to keep the frog method in 1910, 1920 and 1930. Furthermore, he answers that pharmacists claim it would be practically impossible to carry out cat tests since the manufacturers could not obtain enough cats, concluding with a statement that not a single manufacturer voted to adopt the cat procedure.

The U. S. P., 10th revision, makes mandatory a bio-assay for Digitalis, Squill and Strophanthus and their preparations. The method of assay is the one-hour frog procedure with ouabain as the reference standard.

The eleventh revision of the U. S. P. which becomes official June 1, 1936, has as the official method for the assay of Digitalis and its preparations the one-hour frog technique with a Reference Standard Digitalis Powder for comparison. Furthermore, the U. S. P. XI potency requirements are expressed in units directly referable to the International Standard Digitalis Powder.

A consideration of the above list reveals that the official trend of thought, as far as standardization of Digitalis is concerned, favors and even makes mandatory the frog or cat or guinea-pig procedures. It was these methods, therefore, which received emphasis in the report which follows.

PROCEDURE, APPARATUS AND TECHNIQUE.

1. Preparation of the Dilutions.—The frog methods used in this study were: U. S. P. X and modifications (25), U. S. P. XI (26) and the Canadian "Over Night" technique described by Chapman and Morrell (27). The dilutions for injection into the frog were made according to the requirements of the particular method used. Whenever a given preparation was so weak that de-alcoholization was necessary, the required amount of the tincture that was necessary for the dilution was placed in a beaker and a current of air passed over the tincture, after which the calculated amount of water was added. This procedure was always carried out at room temperature.

The dilutions of the tinctures or of the extracts of Digitalis used for cat assays were made up with 0.9 per cent NaCl solution and in no instance did the dilution contain more than 10 per cent alcohol by volume.

2. Apparatus.—The equipment used for the assays was outfitted with a thermoregulating device that maintained a set temperature rigidly and accurately. A very rigid maintenance of temperature is necessary since the rates of absorption are intimately related to the temperature; *vide* Baker (28), Sollmann, Mendenhall and Stingel (29), Roth (30) and Smith and McClosky (31).

3. Procedure.—The frogs received in any shipment were placed in a storage tank and kept there for a period of at least a week before they were used in an assay. The injections and method of observation were followed as directed in the U. S. P. X and U. S. P. XI.

The modifications of the U. S. P. X procedure involved only the time period; that is, in the two-hour and four-hour methods the time only was increased as indicated by the name of the method which appears in the tables.

The cat method that was followed was in all essentials that of Hatcher (23). The procedure differed, however, from the Hatcher-Brody (1) method in two respects: *First*, a supplementary ouabain solution was not used to terminate the test—a Digitalis solution alone being used throughout the assay, and *second*, the duration of the assay was limited to a period of from thirty to forty-five minutes. Burn (32) recommends a thirty- to fifty-five-minute period.

Records of this department show that it matters little what period of time is selected for the duration of the assay (within 30 and 90 minutes) provided that the period of time selected is maintained throughout all the series of assays carried out in any particular study and further provided that a standard of comparison is used in an identical manner, *expressing the potency in*

terms of the reference standard rather than in "animal units." This is an extremely important departure from the usual practice of manufacturers and other workers.

Bio-assay Standards.—The standards used for the frog assays were: Ouabain, the official U. S. P. X standard, the Standard Digitalis Powder of the U. S. P. XI and the Canadian Standard No. 428 (85 mg. equivalent to 100 mg. International Standard) and a second official sample of Canadian Standard (88 mg. equivalent to 100 mg. of International Standard) that was distributed when the supply of Standard No. 428 had been depleted.

The ouabain was kept in a 1:1000 stock solution prepared by dissolving the ouabain in 70 per cent alcohol. Such a stock solution was kept in a refrigerator at a temperature of 1–2° C. except when making the necessary dilutions for the assay. Then the solution was taken from the refrigerator and brought to room temperature before withdrawing the necessary amount. These stock solutions, when prepared and kept as described above, were found to be stable for a year or more, as determined by frequent and careful checks against freshly prepared ouabain solutions.

The standard Digitalis powders, obtained sealed in ampuls of amber glass, were kept in a container in a refrigerator until used.

The simple determination of lethal doses of preparations by the cat method does not indicate reliably the potency of a given Digitalis product. The idea of expressing results in terms of "cat units" was abandoned and in its stead a comparison of unknown Digitalis preparations was made in terms of ouabain and, in the more recent studies of the report, in terms of Standard Digitalis Powders.

PART ONE—STUDY OF TINCTURE DIGITALIS BY THE ONE, TWO AND FOUR-HOUR FROG METHODS AND THE CAT METHOD.

The tinctures of this part of the study were obtained from Dr. J. C. Munch and are described by him (33). Studies and observations made on these samples date from February 1931, so that at the beginning of these studies these samples had been aged for two years. The tinctures were labeled with the following P.T. numbers: Blue A—P.T. 23, Flint A—P.T. 24, Amber A—P.T. 25, Blue B—P.T. 26, Flint B—P.T. 27 and Amber B—P.T. 28. These bottled samples were kept on a laboratory table exposed to diffused light and to the conditions of the room. The results on these preparations are given in Table I.

TABLE I.—COMPILATION OF THE FROG AND CAT ASSAY RESULTS OBTAINED WITH P.T. 23, 24, 25, 26, 27 AND 28.

	U. S. P. X One-Hour Frog.		2-Hour Frog.		4-Hour Frog.		Cat Method.	
Preparation.	Date of Assay.	Ouabain Equiv. of 1 Cc. Mg.	Date of Assay.	Ouabain Equiv. of 1 Cc. Mg.	Date of Assay.	Ouabain Equiv. of 1 Cc. Mg.	Date of Assay.	Ouabain Equiv. of 1 Cc. Mg.
P.T.	8-14-31	0.0550	2-24-31	0.0700	8-15-31	0.0500	3-6-31	0.1057
23	2-10-32	0.0354	8-17-31	0.0454	.....	....	5-27-31	0.0897
	3-15-32	0.0450	2-9-32	0.0347	3-14-32	0.0363	12-4-31	0.0883
	2-27-33	0.0857	3-10-32	0.0438	.....	....	12-20-32	0.1117
P.T.	2-25-31	0.0700	4-17-31	0.0625	4-17-31	0.0625	6-19-31	0.0889
24	4-18-31	0.0613	8-17-31	0.0500	8-15-31	0.0583	12-9-31	0.0906
	8-14-31	0.0523	2-9-32	0.0347	3-14-32	0.0375	12-23-32	0.0912
	2-10-32	0.0344	3-10-32	0.0500	.....	....	.....	....
	3-15-32	0.0430	.....	....	.....	....	.....	....
	2-27-33	0.0800	.....	....	.....	....	.....	....
P.T.	2-25-31	0.0786	4-21-31	0.0635	4-22-31	0.0435	5-1-31	0.1049
25	4-18-31	0.0625	8-18-31	0.0593	8-15-31	0.0573	12-10-31	0.0914
	8-14-31	0.0550	2-9-32	0.0385	3-14-32	0.0438	12-8-32	0.0966
	2-10-32	0.0370	3-10-32	0.0500	.....	....	5-17-34	0.0648
	3-15-32	0.0405	.....	....	.....	....	7-12-34	0.0699
	2-27-33	0.0857	.....	....	.....	....	6-20-35	0.0856
	3-29-34	0.0714	.....	....	.....	....	4-18-36	0.0891
	5-15-34	0.0875	.....	....	.....	....	.....	....
	7-12-35	0.0769	.....	....	.....	....	.....	....
	3-26-36	0.0769	.....	....	.....	....	.....	....

P.T.	3-19-31	0.050	10-24-31	0.0375	10-24-31	0.0375	4-10-31	0.0667
26	10-23-31	0.033	3-31-32	0.0167	4-2-32	0.0200	6-25-31	0.0755
	4-1-32	0.016	.....	....	.....	....	12-17-31	0.0671
							12-27-32	0.0691
P.T.	3-19-31	0.055	3-20-31	0.0450	10-24-31	0.0429	7-11-31	0.0698
27	10-23-31	0.040	10-27-31	0.0364	4-2-32	0.0180	12-18-31	0.0746
	4-1-32	0.019	3-31-32	0.0200	.....	....	12-29-32	0.0722
P.T.	4-18-31	0.0400	3-20-31	0.05	4-22-31	0.025	7-15-31	0.0623
28	10-23-31	0.0375	4-18-31	0.05	10-24-31	0.043	12-23-31	0.0535
	4-1-32	0.0200	10-27-31	0.04	4-2-32	0.020	12-16-32	0.0597
	3-27-36	0.0556	3-31-32	0.02	.....	....	4-28-36	0.0587

DISCUSSION OF TABLE I.

In 1931 and 1932 the preparations were assayed by the U. S. P. X one-hour and the two- and four-hour modifications of the U. S. P. X method and also by the cat method. The cat method as applied in 1931–1932 involved the use of a fixed anesthetic, chloretone. The chloretone in 50 per cent alcohol solution was injected intraperitoneally. During the induction stage the cats were anesthetized lightly with ether. The M. L. D. of ouabain determined on cats anesthetized as just described was 0.1112 mg. per K. of animal. The use of chloretone, or any fixed anesthetic, was abandoned after 1932 and in its stead ether alone was used, the cats being kept lightly anesthetized with this volatile anesthetic. This method of maintaining a rather regular and easily controlled depth of anesthesia was found to be much more practical and convenient than the procedure involving the use of a fixed anesthetic. Cats anesthetized lightly with ether were slightly more susceptible to ouabain and digitalis than when chloretone was used. Average M. L. D.'s determined over several periods of time were as follows: 0.097, 0.094 and 0.095 mg. per K. of animal, using from 5 to 20 cats per assay. Cats anesthetized with chloretone were less sensitive and gave M. L. D. values somewhat greater than 0.1 mg. per K. Bauer and Fromherz (34) in studying the influence of the narcotic on the M. L. D. of ouabain and digitaloids found the M. L. D. of ouabain to be 0.114 mg. and 0.096 mg. per K. of cat when the animals were anesthetized with a barbiturate and "light ether," respectively.

Reference to Table I reveals that by the cat method there was no demonstrable loss in potency in the tinctures. Furthermore, there was no definitely demonstrable difference in potency among the different samples of one series. The A tinctures, however, were definitely stronger than the B samples, whose potency, relative to A on the basis of the dilution made, was estimated quite accurately.

The frog methods revealed, on the other hand, at first a gradual drop in potency in terms of ouabain beginning with the 1931 assays and continuing through 1932. A survey of the results by the frog methods showed that these procedures gave good agreement between the relative potencies of the A and B tinctures but these potencies were very much lower than those shown by the cat assays.

Furthermore, a comparison of the frog methods, one against the other, disclosed that the four-hour technique yielded the more definite and clearer end-point. The absorption from the lymph sac, on the whole, was better than with the shorter

observation periods. The absorption in the frogs used in the shorter periods of assay was irregular and inconsistent; sometimes good and at other times poor.

Further studies on the potencies of these preparations were limited to observations on P.T. 25 because the relative potencies of these samples were definitely established and there was no purpose in repeating tests on similar preparations. The one-hour technique of the U. S. P. X was followed in these latter assays since it was the official procedure, and official opinion pointed to the retention of a one-hour technique in the U. S. P. XI. In the final set of assays, observations on P.T. 28 were included to see whether the 100:70 ratio of potency still held.

The estimations of potency carried out beginning with 1933 showed rather interesting results. The potency of P.T. 25 apparently had risen from a level of approximately 50 per cent U. S. P. X potency to a potency of slightly more than 100 per cent, in terms of ouabain. Assays in 1934 showed potencies of 86 per cent and 105 per cent of U. S. P. X requirements. Cat potencies for the period in 1934 were at a level of 79 per cent and 84 per cent in terms of ouabain, a rather good agreement between the official method and the cat method. In 1935, the frog method showed a potency of 93 per cent, while the cat technique gave a result of 103 per cent; again a good agreement. The 1936 assays disclosed the fact that P.T. 25, by the frog technique, was 93 per cent, and 107 per cent by the cat technique. P.T. 28 was 67 per cent by the frog method and 71 per cent by the cat assay.

TABLE II.

Preparation No.	Date.	M. L. D. of Prep. Cc. Tr.	Std. Deviation from the Mean; + or −.	Ouabain Equivalent of Prep. Mg.	Std. Deviation from the Mean; + or −.
23	3-6-31	1.05	0.04	0.1112	0.0162
	5-27-31	1.24	0.10	0.1112	0.0162
	12-4-31	1.20	0.06	0.106	0.0192
	12-20-32	1.02	0.06	0.114	0.0168
24	6-19-31	1.25	0.09	0.1112	0.0162
	12-9-31	1.17	0.07	0.106	0.0192
	12-23-32	1.25	0.06	0.114	0.0168
25	5-1-31	1.06	0.03	0.1112	0.0162
	12-10-31	1.16	0.10	0.106	0.0192
	12-8-32	1.18	0.09	0.114	0.0168
	5-17-34	1.50	0.16	0.09719	0.0223
	7-12-34	1.39	0.10	0.09719	0.0223
	6-20-35	1.10	0.07	0.0943	0.0052
	4-18-36	1.10	0.08	0.0981	0.0137
26	4-10-31	1.67	0.10	0.1112	0.0162
	6-25-31	1.47	0.09	0.1112	0.0162
	12-17-31	1.58	0.11	0.106	0.0192
	12-27-32	1.65	0.09	0.114	0.0168
27	7-11-31	1.59	0.12	0.1112	0.0162
	12-18-31	1.42	0.06	0.106	0.0192
	12-29-32	1.58	0.21	0.114	0.0168
28	7-15-31	1.78	0.14	0.1112	0.0162
	12-23-31	1.98	0.08	0.106	0.0192
	12-16-32	1.91	0.08	0.114	0.0168
	4-28-36	1.67	0.08	0.0981	0.0137

It is believed that such results offer substantial proof that the frog susceptibility to Digitalis and ouabain is not parallel from a quantitative standpoint,

thus making ouabain a wholly unsuitable reference standard for use in the bioassay of Digitalis preparations by the frog method.

Table II is included as a supplement to Table I and it shows the standard deviation from the average for the cat assays shown in Table I. The calculations were made according to Burn (35).

PART TWO—THE HYDROGEN-ION CONCENTRATION STUDY OF TINCTURE DIGITALIS.

It was decided to undertake a p_H study of the Tincture of Digitalis discussed in Part One in order to observe whether there was any relationship between potency and p_H of these tinctures as they aged.

The procedure used in estimating the hydrogen-ion concentrations of the tinctures was in all essentials that suggested by Biilmann (36) for small quantities of material. The method involved the following system:

Pt	Quinhydrone KCl 0.09*M* + HCl 0.01*M*	Sat'd. KCl	Quinhydrone Unknown Solution	Pt

The capillary electrode vessels recommended and described by Cullen and Biilmann (37) were used as the containers for the solutions of the unknown half cells.

No correction was made for the error caused by the alcoholic content of the galenicals. Smith (38) believes, since it is inconvenient to report results in terms of e. m. f. (which is in reality what is determined), it is customary to calculate p_H using the ordinary constants for water. LaMotte workers stated (private communication) that in the case of such preparations as galenicals calculations of p_H were made without correcting for the error that the alcoholic content plays. The following tables, A and B, summarize the results of these tests.

TABLE A.

P. T. No.	Date.	Series No. 1 p_H.	Date.	Series No. 2 p_H.	Date.	Series No. 3 p_H.	Date.	Series No. 4 p_H.
23	8-21-31	5.39	1-30-32	5.62	4-29-32	5.66	2-9-33	5.18
24	8-21-31	5.66	1-30-32	5.75	4-29-32	5.84	2-9-33	5.75
25	8-21-31	5.69	1-30-32	5.63	4-29-32	5.71	2-9-33	5.55
26	8-22-31	5.22	1-30-32	5.50	4-29-32	5.54	2-9-33	5.03
27	8-22-31	5.65	1-30-32	5.58	4-29-32	5.77	2-9-33	5.73
28	8-22-31	5.74	1-30-32	5.59	4-29-32	5.71	2-9-33	5.59

TABLE B.

P. T. No.	Date.	p_H.	U. S. P. X % Potency.
302	2-10-33	5.51	70
317	2-10-33	5.48	106
303	2-10-33	5.45	110
306	2-10-33	5.02	120
*284	2-10-33	5.09	100
294	2-10-33	5.59	175
309	2-10-33	5.49	105
*110	2-10-33	5.12	140

* N. F. V.

DISCUSSION.

Results of series No. 3 showed on the whole a decrease in acidity when compared with series No. 2, while determinations of the No. 4 group showed an increase in acidity when compared with series No. 3. The differences in p_H among the vari-

TABLE III.—DETERIORATION DATA.

	Fresh Preparations.				
	U. S. P. X Method.	C.-M. Canadian Over Night.		Cat Method.	
Identity of Prep.	Ouabain Equiv. of 1 Cc. Mg.	Can. Std. Powd. Equiv. of 1 Cc. Mg.	Ouabain Equiv. of 1 Cc. Mg.	Can. Std. Powd. Equiv. of 1 Cc. Mg.	Ouabain Equiv. of 1 Cc. Mg.
Tr. B 843	0.109	100.7	0.0481	103.9	0.1117
Tr. A 844	0.100	120.6	0.0576	92.6	0.0996
Tr. Conc. 845	0.444	537.6	0.2560	512.7	0.5514
Tr. F	* Not assayed	120.3	0.0870	96.7	0.1041
	After 6 Months' Aging.				
	U. S. P. X Method.	C.-M. Canadian Over Night.		Cat Method.	
Identity of Prep.	Ouabain Equiv. of 1 Cc. Mg.	Can. Std. Powd. Equiv. of 1 Cc. Mg.	Ouabain Equiv. of 1 Cc. Mg.	Can. Std. Powd. Equiv. of 1 Cc. Mg.	Ouabain Equiv. of 1 Cc. Mg.
Tr. B 843	0.100	73.7	0.043	88.5	0.0990
Tr. A 842	0.100	85.5	0.0498	84.3	0.0942
Tr. Conc. 845	0.417	351.0	0.239	461.4	0.5165
Tr. F	0.1**	66.6	0.0454	86.8	0.0971

* Preparation 4.5 months old when first assayed

** 13.5 months old.

ous preparations were so small as to be of doubtful significance in relation to potency changes.

The observations reported in Table B are interesting, and further the contention that there is apparently no significant relationship between p_H and potency. All the preparations listed in this table were tinctures except P.T. 110 and P.T. 284 which were fluidextracts. P.T. 317 was P.T. 302 fortified by re-percolating more drug with P.T. 302.

In general, the findings reported herein are in agreement with those given by Haag and Jarrett (39), Wokes (40) and Foster and Van Dyke (41).

PART THREE—DETERIORATION DATA ON FOUR SAMPLES OF TINCTURE DIGITALIS.

In this observation four samples were assayed by three methods and their keeping qualities followed. Three samples, P.T. 843, P.T. 844 and P.T. 845, were samples prepared for commercial distribution while the Tincture F was not. The methods of preparation of the first three samples were not available. Tincture F was prepared by collecting 1000 cc. of percolate from 100 Gm. of a Canadian Digitalis Leaf. The U. S. P. X technique was used in preparing this latter tincture.

P.T. 845 was labeled four times tincture strength while the labels of P.T. 843 and P.T. 844 bore no special statements or explanations.

TABLE IV.

First Assays.

Preparation.	M. L. D. of Prep. Cc. per Kg.	Std. Deviation of the Mean; + or −.	Ouabain M. L. D. Mg. per Kg.	Std. Deviation of the Mean; + or −.	M. L. D. of Can. Std. Powd. Mg. per Kg.	Std. Deviation of the Mean; + or −.
Tr. B P.T. 843	0.84	0.063	0.09429	0.005	87.67	5.57
Tr. A P.T. 844	0.95	0.04	0.09429	0.005	87.67	5.57
Tr. Conc. P.T. 845	0.171	0.008	0.09429	0.005	87.67	5.57
Tr. F	0.91	0.04	0.09429	0.005	87.67	5.57

Second Assays.

Preparation.	M. L. D. of Prep. Cc. per Kg.	Std. Deviation of the Mean; + or −.	Ouabain M. L. D. Mg. per Kg.	Std. Deviation of the Mean; + or −.	M. L. D. of Can. Std. Powd. Mg. per Kg.	Std. Deviation of the Mean; + or −.
Tr. B						
P.T. 843	0.99	0.08	0.09805	0.0137	87.67	5.57
Tr. A						
P.T. 844	1.04	0.09	0.09805	0.0137	87.67	5.57
Tr. Conc.						
P.T. 845	0.190	0.001	0.09805	0.0137	87.67	5.57
Tr. F	1.01	0.09	0.09805	0.0137	87.67	5.57

The samples were assayed by: (1) U. S. P. X, (2) Canadian Over Night and (3) cat methods. The Canadian reference standard powder used in this particular study had the following relation in the International Standard: 1.0 Gm. International Standard was equivalent to 0.88 Gm. Canadian Standard Powder. The results of the observations are reported in Table III. Table IV supplements the results in Table III in that it gives the standard deviation of the mean for the cat assays.

DISCUSSION.

The potency relationships existing among P.T. 843, P.T. 844 and P.T. 845 will be considered first. The concentrate was found to be four times the potency of A and B by the U. S. P. X procedure. The cat results for A and B are in agreement with the one-hour results but the cat value of the concentrate was 1.25 times greater than the U. S. P. X frog value and was 5.5 and 5 times the potency of A and B, respectively, when the cat values are referred to ouabain as the standard. The ratio of the potencies by the cat method in terms of the Canadian Standard were the same as when expressed in terms of the cat ouabain values. Therefore, in the case of the concentrate, the cat method revealed a definitely greater potency than the one-hour frog procedure. The Canadian over night technique showed that the concentrate was 5.3 times stronger than B and 4.4 times stronger than A when the results are referred to the Canadian Standard Powder. On the other hand, although the over night ouabain values indicated the potencies of the samples to be in the same ratio as those obtained with the Canadian Standard Powder, they did not agree with the U. S. P. X values. The over night ouabain potencies were from 50–60 per cent of the U. S. P. ouabain values.

These preparations, after standing six months on the laboratory table and under ordinary room conditions, did not show a loss of potency by the U. S. P. X procedure. The cat method showed a slight drop in potency but this loss is insignificant when one considers the margin of error accorded the procedure. By the Canadian technique, A and B deteriorated to 71 per cent and 73 per cent of their original respective potencies; the concentrate assayed 65 per cent of its original potency.

Tincture F could not be compared as fully as the above-discussed preparations since one-hour results on F were not obtained when first assayed because the available stock of frogs had been depleted in obtaining over night results. A comparison of the over night and cat methods, however, reveals again that deterioration by the cat method is insignificant while the over night method sets the potency of F at 55 per cent of its previous potency.

A rather significant point in the above deterioration study is the fact that the drop in physiologic activity for A, B and the concentrate ranged from 65 per cent

to 73 per cent by the Canadian technique, and the concentrate was deteriorating as rapidly as the ordinary tincture.

PART FOUR—THE RELATIVE MERITS OF PROCESSES OF EXTRACTION OF DIGITALIS POWDERS BY MACERATION, PERCOLATION AND SOXHLET EXTRACTION.

1. Residual Activity Studies.—In the course of an assay of a Digitalis preparation for Canadian distribution, it was found that this preparation yielded two entirely different results when assayed in terms of (*a*) the Canadian Standard extracted by Soxhlet for two hours with absolute alcohol, as required in the Canadian specifications (42), and (*b*) the Canadian Standard extracted as in (*a*) but with 95 per cent alcohol. The 95 per cent alcohol gave the more potent Standard extract. This difference in potency initiated an investigation of the Canadian procedure of extraction of Digitalis powders in comparison with other methods. Thus it became the object of this study to determine whether or not the Canadian technique, if rigidly followed, was fully efficient in extracting the total activity of a given Digitalis powder. This information would prove valuable to bio-assayists since manufacturers who distribute Digitalis in Canada standardize these products according to the Canadian procedure. Furthermore, if the Canadian technique does not yield a complete extraction, then the Digitalis preparations assayed and adjusted according to the results obtained by this method, would appear on the market actually weaker than the intended requirements.

The activity of the various extractives prepared was followed by using the regular Canadian Over Night Method of assay (27). At first, the only deviation, and that by necessity, from this technique was in the number of frogs used in estimating the potency of the majority of the residual extracts (q. v. Table V).

TABLE V.—DATA ON RESIDUAL EXTRACTIVES.

Identity of Preparation.	Ouabain Equiv. of Total Ext., after 1st Ext'n Mg.	Ouabain Equiv. of Total Residual Ext'n Mg.	Duration of Residual Ext'n in Hours.	Absolute Ouabain Equiv. of the Digitalis Powd. Mg.	% of Absolute Ouabain Equiv. Remaining after 1st Extraction.	Number of Frogs in the Residual Test.
1 2-hr. abs. ext. powd. P.T. 733 of 3-22-35	0.3320	0.11628	5.0	0.44828	25.9	10
2 2-hr. abs. ext. Can. Powd. of 3-22-35	0.36613	0.09030	5.0	0.45643	19.8	6
3 2-hr. abs. ext. Can. Powd. of 4-26-35	0.33330	0.09263	4.0	0.42593	21.7	10
4 2-hr. abs. ext. Can. Powd. of 5-23-35	0.42625	Negative	2.5	.....	..	10
5 2-hr. abs. ext. Can. Powd. of 6-27-36	0.40000	0.10137	6.75	0.50137	20.2	10
6 2-hr. abs. ext. Powd. No. 432 of 7-16-35	0.52035	0.13215	8.5	0.65250	20.3	5
7 2-hr. abs. ext. Can. Powd. of 7-16-35	0.38675	0.07469	8.5	0.46144	16.2	8
8 2-hr. abs. ext. Can. Powd. of 10-15-35	0.26917	0.04425	6.5	0.31342	14.1	6
9 5-hr. abs. ext. Can. Powd. of 1-7-36	0.49677	0.04092	12.0	0.53769	7.6	10

10	2-hr. abs. ext. Can. Powd. of 1-15-36	0.35900	0.07381	13.0	0.43281	17.5	5
11	2-hr. 95% alc. ext. Can. Powd. of 1-15-36	0.43038	0.04725	13.0	0.47763	9.9	2
12	2-hr. abs. ext. Can. Powd. of 4-15-36	2.06305	0.27380	5.0	2.33685	11.7	25
13	2-hr. 95% alc. ext. Can. Powd. of 4-15-36	2.381652	0.17020	5.0	2.551852	6.7	25
14	2-hr. abs. ext. U. S. P. XI Powd. of 4-15-36	2.885883	0.6355648	5.0	3.521531	18.1	25
15	2-hr. 95% alc. ext. U. S. P. XI Powd. of 4-15-36	3.380872	0.23840	5.0	3.619272	6.6	25

The reason for using less than twenty-five frogs was that the residual extract was obtained from the re-extraction of the small amounts of samples that are ordinarily used in the Canadian method. The samples extracted on 4-15-36 weighed approximately three Gm. or six times the usual weight of a sample used in ordinary routine assay. The purpose of using a larger quantity was: (1) to obtain a volume of residual extract that would provide a quantity sufficiently adequate for twenty-five frogs and (2) to reduce any possible error resulting in losses due to transfer of smaller volumes of extract. The menstruum which was used to extract the powder at first was also used for the re-extraction process.

The residual activity of any given sample was estimated by comparing the activity of the first extract and the re-extracted fraction with ouabain. In this way the total activity of any powder could be computed.

In another phase of this study, the activities of extracts obtained with menstrua other than absolute alcohol were compared. These results are reported in Table VI.

TABLE VI.—COMPARISONS OF EXTRACTION METHODS.

Identity of Preparation.	Date of Assay.	Equivalent of 100 Mg. Can. Std. Powder 2-Hour Abs. Ext. Mg.	% Equivalent of 2-Hour Abs. Ext. Can. Powder.
Can. Powd. 2-hr. abs. ext.	1-7-36	100.00	100.0
Can. Powd. 5-hr. abs. ext.	1-7-36	84.11	118.9
Can. Powd. 2-hr. 95% ext.	1-15-36	84.00	119.0
Can. Powd. 2-hr. abs. ext.	1-15-36	100.00	100.0
U. S. P. XI Std. Powd. 2-hr. abs. ext.	4-15-36	70.70	141.4
U. S. P. XI Std. Powd. 2-hr. 95% ext.	4-15-36	60.00	167.0
Can. Powd. 2-hr. abs. ext.	4-15-36	100.00	100.0
Can Powd. 2-hr. 95% ext.	4-15-36	83.10	120.3
U. S. P. XI Std. Powd. macerate	4-16-36	55.60	179.9
U. S. P. XI Std. Powd. percolate	4-16-36	58.10	172.1
Can Powd. macerate	4-16-36	89.40	111.9
Can. Powd. percolate	4-16-36	101.00	99.0

DISCUSSION.

In this study, the ouabain equivalents of both the first extract and the residual extract were always determined. The sum of the ouabain equivalents of the two total extracts indicates the total physiologic activity of any given sample. The following example is given to illustrate how the residual activity was calculated.

Example: 0.02 cc. per Gm. frog of an aqueous dilution of an extract of powder P.T. 733 caused 50 per cent mortality which corresponds to a dose number of 4.0 (cf. Chapman and Morrell (27) while 0.00035 mg. of ouabain caused 70 per cent mortality corresponding to a dose number of 4.22. Therefore 0.02 cc. is equivalent to:

$$\frac{4.0}{4.22} \times 0.00035 = 0.000332 \text{ mg. ouabain}$$

Since the total volume of this dilution was 20 cc. the ouabain equivalent of the total sample of this aqueous dilution, which represents the total amount of the sample of powder by the first extraction, becomes:

$$0.000332 \times \frac{20}{0.02} = 0.332 \text{ mg.}$$

The aqueous dilution of the residual extract or re-extracted portion in a dose of 0.025 cc. per Gm. of frog caused a mortaility of 40 per cent corresponding to a dose number of 3.9 while 0.00035 mg. of ouabain produced 70 per cent mortality. Thus 0.025 cc. of the aqueous diluted re-extractive is equivalent to:

$$\frac{3.9}{4.22} \times 0.00035 = 0.000323 \text{ mg. ouabain}$$

The ouabain equivalent of the total residual extract becomes:

$$0.000323 \times \frac{9}{0.025} = 0.11628 \text{ mg. ouabain,}$$

since the total volume representing the residual extract was 9 cc.

The true or absolute activity of this particular sample of powdered Digitalis becomes the equivalent of $0.332 + 0.11628 = 0.44828$ mg. of ouabain and the per cent residual activity is represented by:

$$\frac{0.11628}{0.44828} \times 100 = 25.9\%$$

A study of Table V reveals that: (1) 10–25% of the activity remains in the powders extracted with absolute alcohol for two hours, (2) a five-hour extraction with absolute alcohol and a two-hour extraction with 95 per cent alcohol yield extracts of the same potency and (3) the residual activity after a five-hour absolute, and two-hour 95 per cent alcohol extractions is less than 10 per cent of the total potency of the powder.

The period of time that was followed in the re-extraction processes varied as is indicated in Table V. The period of time selected at first for re-extraction was set at five hours since literature reports indicate that six to eight hours give complete extractions. The 1932 Edition of the British Pharmacopœia (43) specifies a six-hour extraction with absolute alcohol in a continuous extraction apparatus. Foster and Van Dyke (41) state eight hours' continuous extraction with absolute alcohol gives a complete extraction. However, they point out that four-hour extracts were not significantly less potent than the eight-hour extract.

Later it was deemed advisable to observe the influence of periods of time less and greater than the five-hour re-extraction period on the residual activity of the

Digitalis powders. The data compiled in Table V indicate that re-extraction of at least four hours is necessary in order to estimate the activity of these residues. Furthermore, the results show that the activity determined for the residual extracts of the powders extracted for five hours with absolute alcohol and those extracted for two hours with 95 per cent alcohol, is of such magnitude as to be insignificant from a standpoint of bio-assay standardization. This indicates that for practical purposes of routine standardization by the Canadian Over Night technique, a five-hour absolute alcohol extract and a two-hour 95 per cent alcohol extract may be considered as representing the full activity of the powder.

As a result of the studies reported in Table V, in which the optimum accuracy of the method used could not be realized because a sufficient number of frogs was not used in most of the instances, it was decided to compare the potencies of Digitalis extracts obtained by (1) the U. S. P. XI process for Tincture Digitalis (slow percolation), (2) maceration as prescribed for the U. S. P. XI Standard Digitalis with the single exception that 10 cc. of menstruum were used for each Gm. of powder taken and (3) Soxhlet extraction with 95 per cent and absolute alcohol. The potency determinations were made by following the Canadian Over Night Method.

After examination of Table VI, one comes to the conclusion that the two-hour period of extraction with absolute alcohol in a Soxhlet apparatus does not permit the realization of a complete extraction. On the other hand, two-hour extraction with 95 per cent alcohol, five-hour extraction with absolute alcohol in a Soxhlet apparatus, maceration and percolation yield approximately 20 per cent stronger extracts. An exception to this was noted in the case of the Canadian Standard Powder extracts obtained by percolation and maceration, in which case the percolate was equal in potency to the two-hour absolute alcohol extract while the macerate was 12 per cent stronger. These results indicate that if extraction in a Soxhlet apparatus is to be followed in preparing an extract for assay by the Canadian method, a five-hour extraction with absolute alcohol or a two-hour extraction with 95 per cent alcohol will give a more complete extraction than the present Canadian procedure. However, it must be pointed out that although the Canadian specifications do not permit a complete extraction, the technique does permit an assayist rigidly adhering to the prescribed steps of the method to obtain a fairly constantly uniform extract. By this last statement, it is meant that the relative potency of a given powder of Digitalis compared with the Canadian standard will be indicated quite accurately since the two powders will be extracted approximately to the same degree. This condition will not hold, on the other hand, when it is a question of standardizing a tincture of Digitalis prepared for commercial distribution, since the extraction factor in this case is eliminated. However, it is possible to obtain check assays on a tincture or fluidextract in terms of the Canadian Standard Powder, but the potency thus obtained will not indicate the true activity of that galenical. A tincture standardized by the present Canadian technique passes into the trade actually weaker than is indicated by the specified requirements. The true or absolute equivalent in mg. of Digitalis Standard of such a tincture may be expressed by the following formula:

$$T = A + \left(\frac{x}{y} \times A\right) \text{ in which}$$

T = The absolute value in mg. of standard

A = The apparent value of mg. of standard as found by the assay

$\frac{x}{y}$ = Fraction representing the residual activity.

2. Study of the Deterioration of the Aqueous Dilutions of the Soxhlet Extracts.—The Canadian technique requires that the extraction and dilution of the extract should be made within twenty-four hours of the beginning of injections. It was decided to obtain data on the potency of the aqueous dilutions as they aged. These aqueous dilutions, aged for periods of time ranging from one week to seven and one-half months, were assayed against freshly prepared extracts of the Canadian standard. The aging aqueous dilutions of the extracts were kept in glass containers well stoppered in a refrigerator at a temperature of 4° C. The results of this study are given in two tables, Table VII and Table VIII.

TABLE VII.—DETERIORATION DATA.

Identity of Preparation.	Age of Diluted Extract.	Equivalent of 100 Mg. of Fresh 2-Hr. Abs. Alc. Ext. of Can. Std. Powder Mg.	% Equivalent of Fresh 2-Hr. Abs. Ext.
Can. Powd. 2-hr. abs. ext.	Fresh	100.00	100.0
Can. Powd. 5-hr. abs. ext.	Fresh	84.11	118.9
Can. Powd. 2-hr. abs. ext.	7.5 months	113.63	88.0
Can. Powd. 2-hr. abs. ext.	6.5 months	129.63	77.0
* Powd. No. 432 2-hr. abs. ext.	5.75 months	179.37	47.7
Can. Powd. 2-hr. abs. ext.	5.75 months	138.88	72.0
Can. Powd. 2-hr. abs. ext.	4.5 months	140.85	71.0

* 85.6 mg. of Powder No. 432 were equivalent to 100 mg. of Canadian Standard Powder when freshly prepared.

TABLE VIII.—DETERIORATION DATA.

Identity of Preparation.	Age of Diluted Extract.	Equivalent of 100 Mg. of Fresh 2-Hr. Abs. Alc. Ext. of Can. Std. Powder Mg.	% Equivalent of Fresh 2-Hr. Abs. Ext.
Can. Powd. 2-hr. abs. ext.	Fresh	100.00	100.00
Can. Powd. 2-hr. 95% ext.	Fresh	84.00	119.0
Can. Powd. 2-hr. abs. ext.	1 week	111.40	89.8
Can. Powd. 5-hr. abs. ext.	1 week	98.43	102.0

DISCUSSION.

From a survey of Tables VII and VIII, it is evident that within a week the aqueous dilutions of the Digitalis extracts show a decrease in potency even when these dilutions are kept at the temperature of the refrigerator. Although actual biologic tests do not show a very appreciable deterioration in these week-old aqueous dilutions, the tests indicate that there is evidence of loss in potency. From the data on hand, it also appears that the deterioration rate reaches an equilibrium level at approximately 70 per cent of the original potency after aging four and a half months. Therefore, the requirements set forth by the Canadian method pertaining to the immediate use of the aqueous dilutions of the Digitalis extracts are perfectly rational.

3. Comparison of Extraction Techniques by the U. S. P. XI and Canadian Over Night Methods of Assay.—In this particular phase of the study of the relative potencies of Digitalis extracts obtained by maceration and percolation (as described in Part A) and Soxhlet extraction, it was decided to pay special attention to the physiologic activity of these extracts in terms of U. S. P. X and U. S. P. XI requirements. The standards for comparison in this series of observations were

ouabain and the macerate of the U. S. P. XI Standard Powder (1 cc. of macerate being equivalent to 100 mg. of this powder). Table IX gives the results of this investigation. Potency estimations by the Canadian Over Night Method are also included in this table for the purpose of comparing the one-hour method against the over night technique. Results by the Canadian technique are likewise referable directly to the macerate of the U. S. P. XI Digitalis Standard.

TABLE IX.—COMPARISON OF EXTRACTION METHODS.

Preparation.	U. S. P. X Method. Ouabain Equiv. of 1 Cc. Mg.	U. S. P. XI Method. U. S. P. XI Std. Powd. Equiv. of 1 Cc. Mg.	C.-M. Canadian Method. U. S. P. XI Std. Powd. Equiv. of 1 Cc. Mg.	C.-M. Canadian Method. Ouabain Equivalent of 1 Cc. Mg.	Cat Method. Ouabain Equivalent of 1 Cc. Mg.
Macerate of U. S. P. XI Powd.	0.1670	100.0	100.0	0.1232	....
Percolate of U. S. P. XI Powd.	0.1670	100.0	95.0	0.1169	....
Macerate of Can. Std. Powd.	0.1428	85.7	62.0	0.0761	....
Percolate of Can. Std. Powd.	0.1428	85.7	55.0	0.0673	....
2-hr. 95% Alc. Ext. U. S. P. XI Powd.	0.1670	100.0	92.0	0.1133	....
2-hr. abs. alc. ext. U. S. P. XI Powd.	0.1250	75.0	78.0	0.0961	....
2-hr. 95% alc. ext. Can. Std. Powd.	0.1250	75.0	67.0	0.0819	....
2-hr. abs. alc. Ext. Can. Std. Powder	0.100–0.125	60–75	55.0	0.0682	....
P.T. 843 Tr. B	0.1000	60.0	37.0	0.0430	0.0990
P.T. 844 Tr. A	0.1000	60.0	43.0	0.0498	0.0942
P.T. 845 Tr. Conc.	0.333–0.500	200–300	195.0	0.2385	0.5165
P.T. 25	0.0714	42.9	27.5	0.0321	0.0891
P.T. 28	0.0555	33.3	19.0	0.0221	0.0587
Tr. F	0.1000	60.0	33.3	0.0388	0.0971
Macerate of Powd. No. 432	0.1375	75.0	...	....	....
Percolate of Powd. No. 432	0.1375	75.0	...	....	....
Macerate of Powd. A	0.0917–0.1100	50–60	...	....	....
Percolate of Powd. A	0.1000	54.5	...	....	....
Macerate of Powd. P.T. 417	0.1053	63.2	...	....	....
Percolate of Powd. P.T. 417	0.1267	66.7	...	....	....
Macerate of Powd. P.T. 632	0.1250	75.0	...	....	....
Percolate of Powd. P.T. 632	0.1250	75.0	...	....	....
Macerate of Powd. P.T. 747	0.150–0.1670	75–100	...	....	....
Percolate of Powd. P.T. 747	0.1180	70.6	...	....	....
Macerate of Powd. Minn. Fol.	0.1670	100.0	...	....	....
Percolate of Powd. Minn. Fol.	0.1540	92.3	...	....	....

DISCUSSION.

Observation of these tests reveals that the maceration technique of the U. S. P. XI (26), two-hour extraction in a Soxhlet apparatus with 95 per cent alcohol and slow percolation yield extracts, in general, of the same potency. However, it must be pointed out that it appears that the macerate is a trifle stronger than the percolate when the potencies of these two types of extracts are considered in the light of both the one-hour and Canadian over night techniques.

In several instances the one-hour frog method did not yield clear-cut results since a range of potencies, rather than any one set potency, was the best that could be obtained. The Canadian technique eliminates the possibility of any personal interpretation, a factor which does arise and will persist as long as the so-called "minimum systolic standstill" is the end-point in the one-hour technique. In the case of comparatively strong and fresh preparations, the end-points as a rule are quite definite and easily discernible. On the other hand, if weak samples or aged samples of tinctures be assayed, then the end reaction is not so clear since stopped hearts (even after "massive" doses) are readily stimulated and will beat, then stop and beat again. Furthermore, with aged samples the absorption is erratic and this factor again complicates the picture. Is the poor absorption due to the inherent properties of an aged sample or of a weak sample which has to be concentrated before a suitable dilution can be made, or is the poor absorption caused by the failure of circulation after the frog has absorbed enough to cause complete heart stoppage?

A comparison of the results obtained by the Canadian technique and those obtained by the U. S. P. XI one-hour method shows that the results by the former method are on the whole lower than the potencies shown by the U. S. P. technique. Comparison of the ouabain equivalents by the over night and one-hour methods reveals that the frog is much more susceptible to ouabain by the over night method. As a consequence of this variation in susceptibility, a discussion of potencies in terms of ouabain equivalents is ruled out.

At this point it is worth while to include a brief summary of the conclusions and recommendations made by the Sub-Committee on Digitalis of the A. D. M. A. as reported in the proceedings (44) of this association. Among their recommendations are two that are quite pertinent: (1) the abandoning of ouabain as a reference standard for Digitalis and (2) the replacement of the M. S. D. one-hour frog technique by an M. L. D. technique of six hours or over night, so as to allow sufficient time for a complete absorption and physiologic effect. This particular A. D. M. A. report (44) is based on a collaborative study carried out by operators in twelve laboratories. Two samples of Tincture Digitalis were used in this study.

In 1935, this same Sub-Committee reported (45) the collaborative efforts of ten laboratories on two samples of Tincture Digitalis. One tincture, Sample B, was prepared by diluting a portion of the sample labeled "A" to two-thirds the potency of A. The tests were carried out by the one-hour M. S. D. and the over night M. L. D. frog methods with Canadian Standard Digitalis Powder No. 428 as a reference standard. The potencies of the tinctures assayed in this collaborative study were reported in terms of International Standard Powder. The results obtained in this study were much more uniform than in the previous year when ouabain was used as a reference standard. The average results of the ten laboratories

by the one-hour method were 94.8 per cent for A and 76.3 per cent for B and by the over night method 97.6 per cent for A and 69.8 per cent for B. Thus by the one-hour procedure B was 80.7 per cent of A and by the over night method B was 70.5 per cent of A. The one-hour results tend to show for B a greater potency than is theoretically possible, if the method of preparing B is kept in mind. This committee in analyzing the compiled data of the ten laboratories reveals that there were from four to six different conclusions that could be mathematically calculated and that it was impossible to say which of two or three of the more likely interpretations was actually correct. This committee cites as an example that the data obtained in testing Tincture A by the one-hour method gave interpretations ranging from 77 per cent to 100 per cent. The possibility of misinterpretation of results obtained by the Canadian technique is negligible if not absent. On the other hand, such a condition does not prevail if the one-hour technique as outlined by either U. S. P. X or U. S. P. XI is followed. The collaborative work carried out under A. D. M. A. auspices points to the adoption of an over night technique since such a procedure gives clear-cut results that are devoid of the difficulty of interpretation of an end-point reaction.

It is interesting from a comparative standpoint to see how the results of this laboratory on these two samples are related to the results reported by the Digitalis Sub-Committee:

Preparation.	A. D. M. A. Av. Result.	Result of the Pharmacology Lab. of the School of Pharmacy.
	ONE-HOUR FROG METHOD.	
Tr. A	94.6%	100%
Tr. B	76.3%	86%
	OVER NIGHT FROG METHOD.	
Tr. A	97.6%	109%
Tr. B	69.8%	75%

The results of this laboratory are in concordance with the general findings of the A. D. M. A. report. By the one-hour method B is 86 per cent of A and by the over night technique B is 68.8 per cent of A. Thus by the over night procedure this laboratory was able to estimate accurately the theoretical potency of B.

Some data obtained with cat assays are included in Table IX for the purpose of a review and limited comparison of the three widely used techniques for Digitalis Standardization. These cat assays were previously discussed in the deterioration studies and therefore no further comments on this particular group of assays will be made.

4. A Comparison of the Potency of Digitalis in Terms of the U. S. P. X and U. S. P. XI Requirements.—This study was undertaken with the view of determining the relative potencies when assayed (1) in terms of the U. S. P. X requirements and (2) in terms of U. S. P. XI requirements. The results of this investigation are reported in Table X.

The reference standards for comparison were ouabain (supplied by the Food and Drug Administration of the U. S. Department of Agriculture) and the U. S. P. XI Standard Digitalis Powder (supplied by the Board of Trustees of the U. S. P. Convention). This U. S. P. Reference Digitalis Powder is 1.34 times as strong as the "U. S. P. Digitalis Unit" which in turn is equivalent to 100 mg. of International Standard Digitalis since 74.5 mg. of the U. S. P. XI Reference Powder are equivalent to one U. S. P. Digitalis Unit.

TABLE X.—COMPARISON OF POTENCIES OF DIGITALIS EXTRACTS IN TERMS OF U. S. P. X AND U. S. P. XI REQUIREMENTS.

Preparation.	% U. S. P. X Potency.	% U. S. P. XI Potency.	Ratio of U. S. P. X Potency to U. S. P. XI Potency.	
Macerate U. S. P. XI Powd.	200	134	1.49	
Percolate U. S. P. XI Powd.	200	134	1.49	
Macerate Can. Std. Powd.	172	115	1.50	
Percolate Can. Std. Powd.	172	115	1.50	
2-hr. 95% ext. U. S. P. XI Powd.	200	134	1.49	
2-hr. abs. ext. U. S. P. XI Powd.	151	101	1.50	
2-hr. 95% ext. Can. Powd.	151	101	1.50	
2-hr. abs. ext. Can. Powd.	121–151	80–101	1.48	(average)
P.T. 843	121	80	1.51	
P.T. 844	121	80	1.51	
P.T. 845	401–602	268–402	1.50	(average)
P.T. 25	86	57	1.51	
P.T. 28	67	45	1.49	
Tr. F	121	80	1.51	
Macerate Powd. No. 432	166	101	1.64	
Percolate Powd. No. 432	166	101	1.64	
Macerate Powd. A	110–133	67–80	1.65	(average)
Percolate Powd. A	121	73	1.66	
Macerate Minn. Fol.	200	134	1.49	
Percolate Minn. Fol.	186	124	1.50	
Macerate P.T. 417	127	85	1.49	
Percolate P.T. 417	152	89	1.71	
Macerate P.T. 632	151	101	1.50	
Percolate P.T. 632	151	101	1.50	
Macerate P.T. 747	181–200	101–134	1.62	(average)
Percolate P.T. 747	142	95	1.49	

A survey of Table X shows that the U. S. P. XI potency requirements are definitely greater than those of the U. S. P. X. By actual assay the average of twenty-six tests revealed that the U. S. P. XI potency is 1.531 times as great as the U. S. P. X requirements.

The Canadian Standard Digitalis Powder used in this particular study had the following relationship: 88 mg. of Canadian Standard are equivalent to 100 mg. of International Standard. Thus this sample of Canadian Powder is 1.136 times as strong as the International Standard. The potency of this Canadian Standard referred to the International Powder through a comparison with the U. S. P. XI Reference Powder and as determined by the U. S. P. XI procedure, was found to be 115 per cent of International Powder potency.

Comparison of the U. S. P. XI Digitalis Standard Powder was made on four different occasions. The following table summarizes these tests:

Date.	M. S. D. of Ouabain Mg. per Gm. Frog.	M. S. D. of U. S. P. XI Std. Powder Mg. per Gm. Frog.
3-26-36	0.00050	0.300
3-31-36	0.00055	0.325
4-4-36	0.00050	0.300
4-7-36	0.00050	0.300

In three of the tests 100 mg. of the Digitalis Standard Powder were equivalent to 0.167 mg. of ouabain or 200 per cent U. S. P. X potency but only 134 per cent in terms of U. S. P. XI requirements. In the other test the ouabain equivalent of 100 mg. of this Digitalis was slightly greater—0.169 mg.

GENERAL DISCUSSION.

The over night frog method is to be preferred to the one-hour frog method because the over night procedure allows sufficient time for complete absorption, a factor of paramount importance in obtaining consistently good results, and thus full physiologic effect. At the same time this method eliminates the personal equation in the interpretation of the end-point effects as is often required in the one-hour technique.

The abandonment of ouabain as a reference standard for Digitalis standardization removes an objection to the U. S. P. X procedure for Digitalis standardization, since Digitalis and ouabain are not identical from a standpoint of absorption rate and frog susceptibility.

The most practical method, a method that should give more uniform results, for the preparation of a Digitalis extract of the reference standard is the maceration technique as described for the Reference Digitalis Powder of the U. S. P. XI (26). The maceration technique requires very little of the assayist's time. The variables incident to a percolation procedure are eliminated if a macerate be prepared for use in the assay. Berry and Davis (46), comparing the relative merits of maceration and percolation for the preparation of Tincture of Digitalis found that maceration was just as effective as percolation.

SUMMARY AND CONCLUSIONS.

1. The expression of potency of Digitalis assayed by the cat method in terms of "cat units" is a practice that should be abandoned.

The cat method should involve direct comparison of the unknown with the reference standard powder in order to eliminate differences in results among different workers who use different types and depths of anesthesia, different perfusion rates, etc. The application of identical technique to both unknown and standard and the expression of potency in terms of reference powder instead of "cat units" would eliminate a serious objection to the routine use of this method.

The cat method in the first portion of this study revealed very little loss in potency in tinctures of Digitalis (ouabain was used then as a reference standard) for a period of approximately five years.

2. The one-hour frog method of the U. S. P. X (with ouabain as the standard) yielded peculiar results in a deterioration study conducted over a period of approximately five years. A consistent decrease in potency of the tinctures followed by an increase in potency was observed.

3. When the deterioration of samples of Tincture of Digitalis was followed by the U. S. P. X, Canadian Over Night Frog and the Cat Methods, the Canadian technique was the only method to reveal a significant loss in potency of tinctures aged for approximately six months. The cat method showed a loss that was not mathematically significant.

4. The unsuitability of ouabain as a reference standard for Digitalis is confirmed.

5. No relationship between the potency and p_H of tinctures could be observed.

6. The Canadian extraction technique does not exhaust the activity of a given Digitalis powder. The activity remaining in the sample represents approximately 20 per cent of the total activity of the powder.

7. Maceration, percolation and a modified Canadian technique of Soxhlet extraction of five hours with absolute alcohol or two hours with 95 per cent alcohol yield quantitative extracts satisfactory for practical purposes.

8. The maceration technique as described in the U. S. P. XI for the extraction of the Digitalis Reference Powder was found to be the most satisfactory extraction process for use in the routine bio-assay of Digitalis.

9. The potency requirements for Tincture Digitalis U. S. P. XI are 1.53 times greater than those of U. S. P. X based on the relative susceptibilities of frogs to ouabain and Digitalis in the spring of 1936.

10. The over night technique of the Canadian method is the most satisfactory frog procedure for routine standardization of Digitalis products.

11. Although the U. S. P. XI and Canadian Tinctures of Digitalis are intended to be equivalent to 100 mg. of International Standard Digitalis Powder per cc., the U. S. P. XI tincture is considerably stronger than the Canadian tincture because of the fact that the Canadian extraction technique does not permit a complete extraction of the reference standard.

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STUDIES OF LECITHIN SOAP.*

I. BACTERICIDAL AND DETOXIFYING EFFECT ON INTESTINAL FLORA.

BY L. G. HADJOPOULOS AND SAUL CASPE.[1]

The detergent properties of soaps have been known since their introduction into domestic use. A definite knowledge of their bactericidal value was gradually acquired at a much later period through the observations of such outstanding workers as Koch (1881), Noguchi (1907), Landsteiner and Erlich (1908), Lanar and Flexner (1911), Nichols (1920) and others.

During the last two decades, the bacteriostatic property of soaps has been intensively investigated and the results obtained can be summarized in the words of Eggerth (1–5):

> 1. That there exists within certain limitations, a direct relationship between the molecular weights of the fatty acids, and the corresponding germicidal properties of their respective soaps;
> 2. That the germicidal properties are more manifest in the higher p_H values of the soaps;
> 3. That the soaps of the various fatty acids exhibit selective germicidal values which can be increased or decreased by substituting the alpha hydrogen with hydroxyl, bromine or sulphydryl-groups.

R. R. Spencer (6) experimentally confirmed the selectivity of certain soaps for certain bacteria, and his interesting work on the visible effects of sodium ricinoleate in completely dissolving bacterium tularense, bacillus typhosus, bacillus pyocaneus, bacillus alkaligenes, can be used as a working hypothesis of the mode by which soaps act upon bacteria.

Larson (7) assumed that, in general, these intrinsic properties of soaps are dependent upon their surface tension reducing values. However, he was not able to account by this assumption for the effectiveness of soaps upon bacterial by-products

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* Presented before Scientific Section, A. PH. A., New York meeting, 1937.

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of the nature of exotoxins, and therefore postulated that this latter activity may be referred to the difference between the electric charge of the soap and toxin molecule.

Based on these observations, Larson suggests that

> "The biliary soaps undoubtedly play an important rôle in detoxifying the bacterial toxins of the intestinal tract. The intestinal tract is essentially a thirty-foot culture tube which harbors a large variety of bacteria, many of which growing under favorable conditions, undoubtedly secrete toxins. Had nature not provided an efficient detoxifying mechanism in the form of bile soaps, the host probably would not have survived."

The therapeutic management of intestinal antisepsis and detoxification by use of soaps is predicated upon these principles.

In 1928, we repeated some of the work reported by Eggerth and Larson, and noted a limitation to the general use of soaps as intestinal antiseptics: soaps are irritating to the gastrointestinal mucosa. In an attempt to overcome this disadvantage, we reduced the high alkalinity of the soaps to almost the neutral point, only to witness the fact already noted by Eggerth that these neutral soaps were of diminished therapeutic value.

By administering soaps in the form of pills coated with salol or protected by keratine, we might avoid the undue irritation to the gastric mucosa, especially in cases of hypoacidity and achlorhydria; but our experience with pills was highly unsatisfactory as such a compact mass of soap would invariably escape the intestinal tract without a sufficient amount of emulsification.

Our problem was, therefore, to derive a soap which would pass the gastric mucosa untouched but would readily emulsify in the intestines with the least possible irritation to both. In other words, a soap equally or sufficiently potent at a low p_H, easily taken by mouth in the form of a finely triturated powder and readily emulsified in the intestines.

In the quest of a product with the above qualifications, we soon came to the conclusion that we had to limit our sources to a small group of fatty acids as the most readily emulsifiable bases for soaps. The soaps of the fat-like substances derived from tissue extracts (lipids, lecithids) were found to answer the above qualifications.

The bactericidal selectivity displayed by the ordinary fatty-acid soaps was also noticed in the normal sodium salts of such extractives. Thus, heart tissue extracts (lipids) were more active against the gram-positive group of cocci (even *Streptococcus Fecalis*) while the liver and kidney extracts were more active against the gram-negative paracolon group of bacilli.

In Table I we give in a condensed form a list of experiments to demonstrate the superiority of lipin to ordinary soaps in their relative immediate and remote effects on intestinal flora.

After having chosen as our control a man with no acute or chronic symptoms referable to the gastro-intestinal tract, except a moderate degree of constipation, we made repeated bacteriological examinations of his stools to determine his basal intestinal flora. The results of his final stool examination on 1/15/29, which was in no way different from the previous findings, is given in the first line of Table I.

TABLE I.—THE COMPARATIVE BACTERICIDAL PROPERTIES OF VARIOUS SOAPS ON THE INTESTINAL FLORA.

Date, Medication.	Type of Soap.	Stools Examined.	*Streptococcus.* Hemol.	*Streptococcus.* Virid.	Colon Group Bacillus.	Effect on the G. I. Mucosa.
Control	No medication	1/14/29	0	Innumerable	Innumerable	Normal
1/15/29	Na Lecithid	1/16/29	0	0	0	No unpleasant effect
		1/17/29	0	0	0	....
		1/18/29	0	0	0	....
		1/19/29	0	0	0	....
		1/20/29	0	0	Innumerable	....
		1/21/29	0	500	Innumerable	....
2/6/29	Ord. Comm. Soap	2/7/29	1	10	Innumerable	Slight irritation
		2/8/29	0	1	Innumerable	Rather constipating
		2/9/29	0	2	0	....
		2/12/29	0	11	Innumerable	....
2/17/29	Na Ricinoleate	2/18/29	0	5	15	Markedly irritating
		2/19/29	0	0	50	....
2/20/29	Na Ricinoleate	2/21/29	0	3	15	....
2/22/29	Na Ricinoleate	2/23/29	0	1	400	....
3/19/29	Na Stearate	3/20/29	0	4	20	Slightly irritating
		3/21/29	0	37	4	Rather constipating
		3/22/29	0	500	Innumerable	....
6/17/29	Na Oleate	6/18/29	15	100	Innumerable	Slightly irritating
		6/19/29	21	5	200	....
		6/20/29	0	30	3	....
2/5/30	Na Palmitate	2/6/30	0	1	Innumerable	Fairly well borne
		2/7/30	0	50	Innumerable	....
		2/8/30	0	450	Innumerable	....

COMMENTS: The results, as tabulated, were the result of a single 5-grain dose of the particular soap administered by mouth. We made an exception to this rule in the case of Sod. Ricinoleate for reasons to be mentioned later.

He then submitted to the various soap treatments at sufficient time intervals so as to eliminate the possibility of the effects of one treatment running over to the other. The bacteriological column in Table I was divided into *Streptococcus* (98% *Str. Fecalis*) and *B. Coli*. The *Streptococci* were further differentiated into Hemolytic and Viridans or Anhemolytic. We made no further attempt to differentiate the *Coli*-bacillus group. The number of bacteria was determined from pour blood plate colonizations: a small loopful of stools was thoroughly emulsified in one cc. of neutral broth and mixed in ten cc. of nutrient agar with human blood from which the plates were poured.

Of all the soaps tested the best borne and the most effective clinically was the Lecithid product. Its effect was immediate and uniform, keeping the stools sterile for four days. On the fifth day *B. Coli* reappeared and on the sixth the *Streptococci*. Of the other soaps, the palmitate, the next best borne by mouth, was but weakly active on *Streptococci* and completely inactive for *B. Coli*. The stearate showed a fair degree of activity on both flora, but its effect was of shorter duration. Besides, it manifested a moderate degree of intestinal irritation and tendency toward constipation. Sodium Oleate, the unsaturated fatty acid soap, was probably the most satisfactory of the pure fatty acid group of soaps, although it could not sterilize

completely the stools even for one day. The appearance of hemolytic *Streptococci* during its administration, a fact for which we have no explanation, was surely an undesirable complication. In the case of commercial soaps, the source of which is generally animal and vegetable fats of a mixed fatty acid composition, the results were within ordinary expectations. Finally, Sod. Ricinoleate displayed a fair degree of activity on both intestinal flora, but it was markedly irritating to gastro-intestinal mucosa. In an attempt to fortify and prolong its action, which as one will notice in our table, is not very strong, we administered it every other day to a total of 15 grains. The results, however, were not any different from those of the first dose.

Having thus convinced ourselves about the superiority of Lecithid soaps to others we next endeavored to test its effectiveness in chronic intestinal disorders of

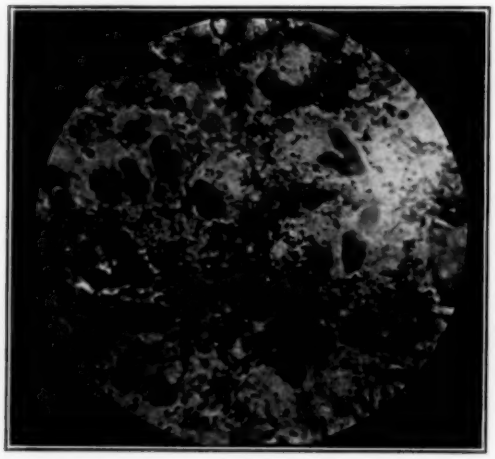

Fig. 1 shows the condition of stools a day before Na Lecithid was administered. Note the abundance of mixed bacterial flora, especially the diplococci and streptococcic chains. The rest of the fecal material is made up mostly of mucous plugs, erythrocytes and a few degenerated and swollen epithelial cells.

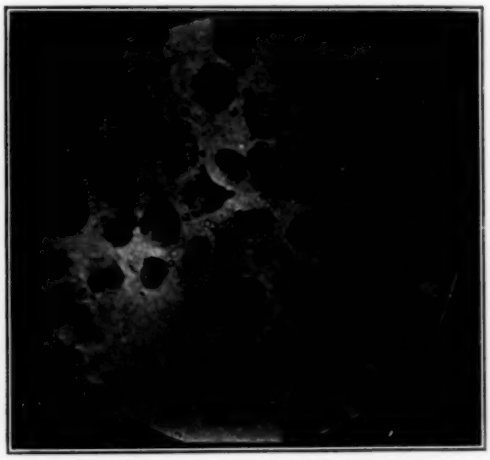

Fig. 2.—A day after medication the stool takes a different aspect. The number of bacteria is reduced to an occasional diplococcus or chain mostly engulfed within phagocytic leucocytes and epithelial cells. Mucous plugs are practically absent and erythrocytes less in number.

the general type where *B. Acidophilis* implantations are clinically recommended. As such cases, we then had at our command a long list of chronic sufferers from Rheumatoid and Hypertropic Arthritis. We instituted this treatment in over a hundred (now over a thousand) cases administering once a week 2 grains of Na Lecithid triturated in 5 grains of Lactose and 1 grain of Phenolphthalein. Clinically our results on the whole were very satisfactory but bacteriologically we were somewhat disappointed in finding out that a quick sterilization of the stools, which was so readily accomplished in normal individuals, was still a problem in these chronic sufferers. It usually took weeks and often months before we could witness a sterile stool in our patients.

To avoid discrimination and bias in selecting cases for report, we tabulated them alphabetically and in Table II we give the first 15 of a list of 100.

TABLE II.—THE EFFECT OF Na LECITHID ON INTESTINAL STREPTOCOCCI IN ARTHRITICS.

Serial Number.	Name Abr.	Treatment Instituted.	Number Colonies per Plate.	Date Bactr. Improvement.	Number Colonies per Plate.
1	App.	4/23/30	Innumerable	10/17/30	1
2	Aus.	4/29/30	Innumerable	9/23/30	20
3	Bru.	8/5/30	Innumerable	11/4/30	0
4	Boy.	5/21/30	Innumerable	10/31/30	0
5	Brug.	1/27/30	Innumerable	11/4/30	0
6	Big.	4/21/30	Innumerable	10/22/30	0
7	Bel.	4/8/30	200	10/16/30	0
8	Bla.	12/18/29	Innumerable	2/6/30	9
9	Bur.	5/5/30	Innumerable	9/30/30	8
10	Bar.	3/18/30	Innumerable	6/11/30	0
11	Bell.	3/11/30	Innumerable	5/28/30	68
12	Bonn.	10/23/29	Innumerable	5/8/30	14
13	Bec.	11/21/29	Innumerable	3/11/30	19
14	Cla.	6/2/30	Innumerable	10/3/30	4
15	Cut.	5/7/30	Innumerable	9/19/30	6

COMMENTS: In this group of chronic Arthritics the outstanding clinical feature, as stated by the patients, was the marked relief from symptoms of auto-intoxication. They were more active and alert, both bodily and mentally, were capable of doing more work efficiently. Jointwise, however, we could not draw any definite conclusions, as we were not solely depending on one particular treatment but a combination of all accepted methods to control the disease.

The most promising field for the therapeutic usefulness of Lipid-soaps and a more direct means of evaluating its proper field of activity would be the use of it in active infections of the intestines such as enterocholitis, summer diarrhoea, etc. Our experience in this respect being very limited (3 cases of enteritis and 5 cases of chronic enterocolitis) we reservedly make the statement that Na Lecithids have a useful field of activity in such ailments of the intestinal tract. We reproduce here three photomicrographs of the stool smears of one of our enteric cases.

Fig. 3.—Shows the condition two weeks later with reappearance of some of the normal stool flora and an occasional diplococcus or chain. The persistence of pus cells is indicative of the fact that the disease is by no means yet cured.

CONCLUSIONS.

1. Lecithin soap manifests specific bactericidal properties upon intestinal *Streptococcus* and *B. Coli*.

2. Lecithin soap is useful as a detoxifying agent and intestinal bactericide in arthritic cases.

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THE ACTION OF CARDIAC DRUGS UPON THE CHROMATOPHORES OF TOADS.*,[1]

BY CHESTER C. HARGREAVES, WILLIAM T. WINCHESTER AND K. K. CHEN.

In the assay of some cardiac substances in the nebulous toad, *Bufo valliceps* (1) (2), it was noted that a pallor of the dorsal skin occurred. Since this phenomenon has not been mentioned by previous workers, it may prove of interest to describe it in detail. Figure 1 illustrates the appearance of a toad injected with

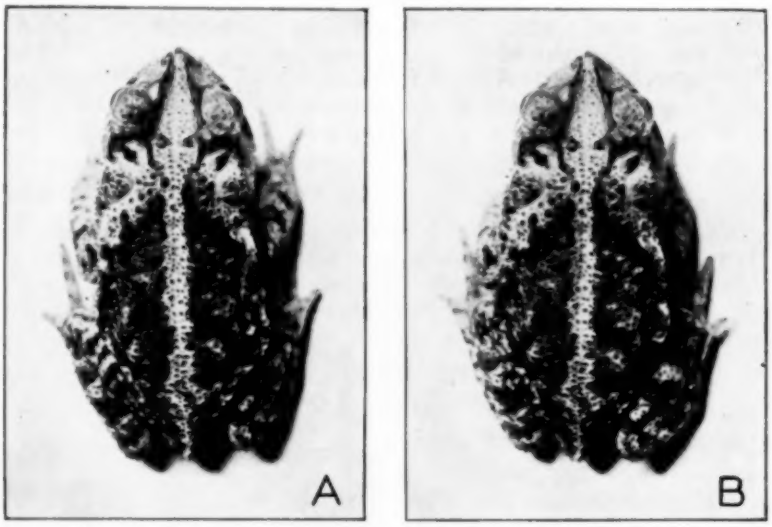

Fig. 1.—Color comparison between a couminginized toad and a control.

A. Control kept under the same conditions as B.

B. A toad was injected with coumingine hydrochloride, 0.175 mg. per Gm. The photograph was taken 40 minutes after the injection. The contrast in density between *A* and *B* is actually more pronounced than these pictures show.

coumingine hydrochloride as compared with that of a control animal. The difference in color between the two toads can be readily made out. To date, four cardiac drugs—ouabain, scillaren B, cymarin and coumingine hydrochloride—have been tested on toads, and apparently they all cause contraction of chromatophores. The reaction is definite with minimal systolic doses of each substance. Thus, the toad turns pale upon the injection into the lymph sac of the following doses: ouabain, 0.05 mg. per Gm.; scillaren B, 0.03 mg. per Gm.; coumingine hydrochloride, 0.175 mg. per Gm.; and cymarin, 1.1 mg. per Gm. Larger amounts, of course, produce the same effect. The change in color from dark to light usually takes place about 15 minutes after the administration of the drug, and becomes more evident 20 to 30 minutes later. The observation is best made when the toads are placed in perforated metal cages, away from any bright light. Rarely is this paleness absent following any of the drugs in the dosage stated. The cause of this change

* Presented before the Scientific Section, A. PH. A., New York meeting, 1937.

[1] From the Lilly Research Laboratories, Indianapolis, Indiana.

in coloration has not been determined. It is uncertain whether it may be a direct action upon the pigment cells, or a secondary effect resulting from the cardiac derangement under the influence of the drugs.

SUMMARY.

Ouabain, scillaren B, coumingine hydrochloride, or cymarin, when injected into the lymph sac of the nebulous toad, *Bufo valliceps*, causes noticeable blanching of the dorsal skin. The reaction is definite with the minimal systolic dose of each cardiac drug.

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THE CHEMISTRY OF PASSIFLORA INCARNATA.*

BY EDWIN J. FELLOWS[1] AND CLAYTON S. SMITH.**

INTRODUCTION.

Passiflora incarnata, one of about 125 species comprising the passion flower family, is a perennial climbing shrub found thriving in dry soils from Virginia to Florida and westward to Missouri and Arkansas. This drug has been used in medicine for almost one hundred years. While the drug is not found in either the current U. S. P. or N. F. it was recognized by N. F. V. There are at the present time, however, numerous hypnotic and sedative preparations of *Passiflora incarnata* on the market.

Fisher (1), the first to report on the clinical use of *Passiflora incarnata* stated that it was to be preferred to the bromides and chloral as a sedative, antispasmodic and hypnotic because the sleep it induced more closely approximated natural slumber. Sayre (2) in 1902 reported that passiflora had gained favor among practitioners in the treatment of insomnia, although there was some controversy as to its value. Three years later Stapleton (3) reported the successful use of tincture of passiflora in cases of insomnia from hysteria, neurasthenia and neuralgia. Leclerc (4) later reported the use of a tincture containing 50 per cent alcohol as a hypnotic and sedative with some degree of success. In the same year Hinsdale (5) reported demonstrations of a sedative action by means of reaction time experiments on human subjects. In the following year Hinsdale (6) reported diminution of acuteness of hearing of a number of human subjects after administration of tincture of *Passiflora incarnata*.

Discussions (7) concerning the components of passiflora are either to the effect that nothing is known about its chemical composition or that it is supposed to

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* Presented before the Scientific Section, A. PH. A., New York meeting, 1937.

** We are indebted to Eli Lilly & Co. for their generous coöperation in furnishing the crude drug used in this investigation.

[1] Abstracted from a dissertation submitted June 1935, by Edwin J. Fellows in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Ohio State University.

contain an alkaloid. A search of the literature has failed to disclose satisfactory evidence that the drug contains an alkaloid. Ott (8) in 1898 reported that the drug was a depressant to the motor side of the cord, increased the respiratory rate and momentarily lowered blood pressure in experimental animals. Pilcher (9) reported that the drug has no effect on excised uteri of guinea pigs. DeNito (10) investigated several species of passiflora other than incarnata and it was his belief that they contained an alkaloid. The same author (10) also carried out a number of biological tests on a fluidextract of passiflora. Very little has been done toward standardization of *Passiflora incarnata*. In 1916 the Committee on Standards of the AMERICAN PHARMACEUTICAL ASSOCIATION stated that passiflora should not contain over 12 per cent ash. In N. F. V the standard was fixed at not more than 5 per cent of stems over 8 mm. in diameter.

This survey of the available literature on the subject discloses the fact that *Passiflora incarnata* has received wide-spread clinical application and that its continued use rests solely on an empirical basis. There also is a lack of reliable information concerning the chemical components of the drug. In order to secure some definite information concerning the chemical constituents of this drug the work described in the present paper was undertaken.

Analysis of Crude Drug.—To serve as a guide in subsequent experiments a proximate analysis was made. The results of these determinations have been summarized in Table I.

TABLE I.

Determination.	Per Cent.
Moisture (1)	7.60
Total ash (2)	15.60
Water-soluble ash	3.70
Water-insoluble ash	11.90
Acid-soluble ash	10.33
Acid-insoluble ash	5.25
Water-soluble constituents	25.40
Crude fiber (3)	29.20
Protein (4)	11.30
Petroleum ether extract	1.80
Ethyl ether extract	2.20
Absolute alcohol extract	1.79

Method Used: (1) Crude drug dried to constant weight at 100° C.; (2) Woodman Food Analysis, 2nd Edition, page 16; (3) U. S. P. X, page 465; (4) total nitrogen × 6.25.

Extraction of Drug with Organic Solvents.—Approximately 30 kilos of finely ground drug were completely extracted at room temperature with diethyl ether for a period of two weeks. This extract was reduced to a solid by distillation under reduced pressure and the residue so obtained completely extracted with petroleum ether. The portion of the crude drug insoluble in ethyl ether was air dried and then completely extracted with absolute alcohol.

The petroleum-ether extract was distilled under reduced pressure until practically all of the solvent had been removed and then the residue remaining in the distilling flask steam distilled. A volatile oil was obtained but the amount was insufficient to permit purification or a detailed examination. The constants obtained for this oil are listed in Table II.

TABLE II.

Specific Gravity, 27° C.	Specific Rotation, 27° C.	Refractive Index, 27° C.
0.810	+16	1.4382

The material not volatile with steam was saponified and after adding a large volume of water to insure complete solution of the soaps, extracted with ethyl ether. The ether extract was set aside for future investigation. The acids obtained by hydrolysis of the saponified material were esterified by heating under a reflux condenser for 18 hours with a large excess of methyl alcohol containing 2 per cent dry HCl gas. The esterified material was decolorized with Norite and the esters subsequently distilled at 5-mm. pressure, separated into two fractions and the following constants determined.

	B. P. (5 Mm.). ° C.	Mean Mol. Wt. (Acids).	Iodine Number (Esters).
Fraction I	170–200	259	126
Fraction II	200–255	283	109

These two fractions were combined, fractionally distilled three times at 15-mm. pressure and fractions collected at each 5° interval between 185° and 220° C. Throughout the distillations a constant pressure was maintained by a device similar to the one described by Huntress and Hershberg (11) and the distillates collected in a modified Pauly receiver (12). The analytical data for the various fractions are to be found in Table III.

TABLE III.

Fraction Number.	B. P. Me Esters, 15 Mm. ° C.	M. Mol. Weight (Acids).	Iodine Number (Esters).	Fraction Weight (Gm.).
1	185	254	29	2
2	185–190	262	38	8
3	190–195	266	66	10
4	195–200	272	92	18
5	200–205	274	131	12
6	205–210	276	149	28
7	210–215	287	169	13
8	215–220	305	161	3
9	Above 220	574	118	8

The data in the above table indicate two things: *First*, that acids of the C_{18} series predominate (Mean Mol. Wt., B. P. and fraction weights); *second*, that unsaturated fatty acids are present in all fractions (iodine numbers). Each of the fractions in this table was subjected to the lead salt ether method described by Jamieson (13). The data obtained by analysis of the acids resulting from the decomposition of the lead soaps insoluble in ether are to be found in Table IV.

TABLE IV.—SATURATED ACIDS OBTAINED BY LEAD SOAP ETHER SEPARATION.

Fraction Number.	M. Mol. Weight.	Iodine Number.	Fraction Weight (Gm.).
1	240	10	0.8
2	242	11	5.0
3	247	13	6.0
4	253	15	7.0
5	257	16	4.0
6	262	15	7.0
7	279	14	1.0
8	...	..	0.1
9	362	36	0.7

Since the molecular weight of palmitic acid (C_{16}) is 256, it is probable that an acid or acids of lower molecular weight are present in Fractions 1, 2 and 3 of Table IV. The presence of acids of the C_{16} series appears to be clearly indicated from the mean molecular weight data for fractions 4, 5 and 6. Fraction 7, however, probably contains a mixture of acids of the C_{16} and C_{18} series. The data for Fraction 9 indicate the presence of an acid above the C_{18} series or of material other than fatty acids.

Fractions 1, 2 and 3 were combined and fractionally crystallized from acetone. After repeated crystallization a compound melting at 54° C. and having a mean molecular weight of 235

was obtained. This should establish the presence of myristic acid, m. p. 53.8° C., molecular weight 228.

Fractions 4, 5 and 6 in Table IV were combined and repeatedly crystallized from acetone. A product was obtained which melted sharply at 62.2° C. and had a mean molecular weight of 257. The following data indicate that the product is palmitic acid:

	M. P. ° C.	M. Mol. Wt.	Ultimate Analysis, Per Cent. C.	H.
Palmitic acid	62.6–63.0	256.3	75.00	12.00
Sample	62.2	257.0	75.21	12.40

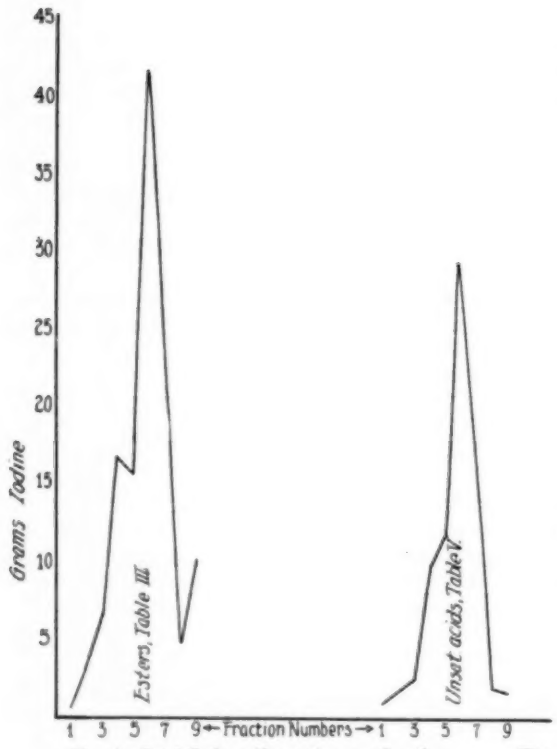

Fig. A. Total Iodine Absorption per fraction.

No depression of the melting point was observed when the product was mixed with an authentic sample of palmitic acid. The small amount of material available in Fractions 7, 8 and 9 prevented further attempts at purification.

The acids obtained by decomposition of the lead soaps *soluble* in ether next were examined. The results are to be found in Table V. The mean molecular weight data for Fractions 3, 4, 5, 6 and 7 of Table V indicate that they are made up almost entirely of acids of the C_{18} series. The iodine numbers of these fractions show the presence of acids with more than two double bonds. The mean molecular weights for Fractions 1 and 2 indicate the possible presence of unsaturated acids below the C_{18} series.

The total iodine absorption values for the fractions in Table III as well as for those in Table V have been plotted against the fraction numbers in Fig. A. These values have been calculated by the formula

$$\text{Iodine absorption} = \frac{\text{Wt. of Fraction} \times I_2 \text{ number}}{100}$$

TABLE V.

Fraction Number.	M. Mol. Weight.	Iodine Number.	Fraction Weight (Gm.).
1	266.0	98.6	0.3
2	267.0	114.0	1.4
3	273.0	183.0	1.3
4	276.5	192.2	5.0
5	279.5	193.0	6.0
6	282.0	194.0	15.0
7	284.5	183.0	7.0
8	292.0	167.0	1.0
9	352.5	150.0	1.0

Inspection of the curve shows a peak for Fraction 6 of both Tables III and V. The mean molecular weight for Fraction 6, Table III, is 276; and for Fraction 6, Table V, 282. This indicates the presence of unsaturated fatty acids containing 18 carbon atoms. Likewise lesser peaks at Fraction 4 in each table indicate the presence of unsaturated fatty acids containing 16 carbon atoms.

Each of the fractions of Table V was subjected to the barium-soap benzene method of separation for unsaturated fatty acids. Only traces of barium soaps insoluble in benzene were obtained. Table VI contains the analytical data for acids obtained by decomposing the barium soaps *soluble* in benzene. It is to be noted that the iodine numbers of several of the fractions have been slightly raised. Fractions 1–3, inclusive, contained insufficient material to warrant a satisfactory separation by the barium soap benzene method. Inasmuch as Fractions 4, 5, 6 and 7, of Table VI, showed no very marked differences in their analytical constants, they were combined and the polybromide number determined and found to be 26.2. The amount of bromine in the insoluble bromide was determined by the Parr bomb method and found to be 62.92 per cent. The melting point of this compound was 179.5–180.5° C. When these data are compared with similar data for hexabromostearic acid they are in close agreement indicating that the fatty acid before bromination was linolenic acid.

	M. P. ° C.	Bromine, Per Cent.
Hexabromostearic acid	180–181	63.3
Sample	179.5–180.5	62.92

TABLE VI.

raction Number.	M. Mol. Weight.	Iodine Number.
4	282	201
5	280	203
6	279	209
7	284	190
8	295	170
9	349	153

The ether solution and washings from the insoluble bromide were combined and the excess bromine removed with sodium thiosulfate solution. An ether-soluble petroleum-ether insoluble compound was eventually obtained and found to melt at 114° C. Its bromine content by the Parr bomb method was 54.35 per cent. These data compare favorably with those for tetrabromostearic acid as noted in the table below.

	M. P. ° C.	Bromine, Per Cent.
Tetrabromostearic acid	114–115	53.30
Sample	114	54.35

It may, therefore, be concluded that linoleic acid was present in the original fat. The final petroleum ether filtrate from the bromine precipitation may contain some oleic acid since the barium soap benzene method is far from quantitative. In order to establish the presence or absence of oleic acid in the original fat, 5 Gm. of the fatty acids from Fractions 4, 5, 6 and 7, after saponification with NaOH, were oxidized with $KMnO_4$ according to the method of Lapworth and Mottram (14). That oleic acid was present in the original fat is shown by the constants obtained for the oxidized compound.

	M. P. ° C.	M. Mol. Weight.
Dihydroxy-stearic acid	131–132	316
Oxidized compound	130	317

Non-Saponifiable Material.—Any non-saponifiable material present in the fixed oils of the drug would be found in the ether extract after saponification (page 567). After removing the ether from this extract by distillation under reduced pressure a deep red-colored residue remained in the

distilling flask. An attempt was made to dissolve this residue in 95 per cent alcohol. A portion of this material was soluble in cold alcohol, part of the cold alcohol-insoluble material was soluble in hot alcohol and the remainder insoluble in both hot and cold alcohol.

An orange-colored precipitate was obtained on allowing the *hot alcohol-soluble fraction* to cool. After repeated recrystallization from hot alcohol and after drying in a vacuum desiccator over sulfuric acid a nearly white substance melting rather imperfectly between 70–75° C. was obtained. A portion of this purified solid was soluble in hot acetic anhydride, the remainder floated on top the anhydride in the form of oily droplets. On cooling, the hot acetic anhydride soluble material settled out. This suggests the presence of an alcohol (or sterol) and a hydrocarbon in the mixture. Since the substance soluble in hot acetic anhydride gave negative Lieberman-Burchard and Salkowski tests the absence of unsaturated alcohols or sterols is indicated. From this it may be presumed that the material was a mixture of a saturated alcohol and a hydrocarbon. The amount of material available was too small to permit further purification.

The cold alcohol-soluble portion of the non-saponifiable material was subjected to distillation under reduced pressure. After the liquid had been reduced to about one-half its volume and cooled, a mass of rusty colored crystals appeared. After numerous recrystallizations from alcohol white crystalline material was obtained and found to melt at 139° C. This substance had a specific rotation of −34.9. The acetyl derivative (17) of this compound was prepared and after purification was found to melt at 130° C. The following data indicate that the purified precipitate was the sterol sitosterol.

	Salkowski and Lieb.-Burch.	M. P. ° C.	M. P. of Acetate ° C.	$[\alpha]_D^{19°}$.
Sitosterol	#	138–139 (16)	130–131 (16)	−36.6 (16)
Precipitate	#	139	130	−34.9

The non-saponifiable material insoluble in hot alcohol (page 569) separated as a heavy red liquid when suspended in hot alcohol but on cooling hardened into a waxy mass. This was dissolved in acetone from which it crystallized as a light fluffy mass. After numerous recrystallizations from ethyl acetate a nearly white product was obtained which melted at 65–67° C. and when heated with acetic anhydride floated on top the mixture as an oily layer. This material was subjected to the treatment recommended by Leys (18) for hydrocarbons. A compound was obtained by this method which after purification melted between 67–68° C. The purified compound was insoluble in cold concentrated sulfuric acid and was unaffected by fuming nitric acid, did not react with acetic anhydride nor did it give positive tests for sterols. Furthermore, it did not decolorize 1 per cent $KMnO_4$ nor absorb bromine. From these data it may be concluded that a hydrocarbon was present. The following hydrocarbons have been found in plants: Triacontane, m. p. 67° C.; hentriacontane, m. p. 68° C.; dotriacontane, m. p. 70° C.

It will be recalled that the crude drug was first extracted with ethyl ether, the ether removed and the residue remaining extracted with petroleum ether. The portion of the residue insoluble in petroleum ether now will be considered. This residue was extracted with several liters of ethyl ether. Some of the material, which had apparently been carried along in the original extraction of the crude drug, failed to go into solution. After repeated washing with 95 per cent alcohol a nearly white solid was obtained. This was saponified with alcoholic KOH and the saponification mixture extracted with ether. An ether-soluble compound was obtained and purified by recrystallization from ethyl acetate. It was found to melt at 86° C. The compound was completely soluble in acetic anhydride and according to Lewkowitsch (15) should be classified as an alcohol. An acetyl derivative melting at 73° C. was prepared. The analytical constants for this non-saponifiable lipid indicate the presence of melissyl alcohol. The latter substance melts between 85–88° C. and its acetyl derivative at 73° C. An attempt was made to isolate and identify a fatty acid from the saponification mixture. A small amount of material was recovered but due to the inadequacy of the sample the nature of the acid or acids could not be determined. Although a fatty acid was not isolated the presence of melissyl alcohol suggests that the original substance may have contained a wax.

The *original ether extract of the crude drug* insoluble in petroleum ether was extracted with hot water and the aqueous extract steam distilled. A distillate which was acid in reaction was ob-

tained. This was extracted with ether and after evaporation of the ether a residue was obtained which gave all the tests (19) said to be indicative of formic acid. The material remaining in the distilling flask (water-soluble-non-volatile) gave a negative Molisch test and failed to reduce Fehling's solution. After hydrolysis with HCl the solution again failed to reduce Fehling's solution. This eliminates glucosides or other carbohydrate material.

The portion of the *original ether extract* of the crude drug *insoluble in hot water* next was examined. This was extracted with ethyl alcohol in which it was only partially soluble. The *soluble portion* was concentrated to a small volume and allowed to stand in a refrigerator over night. A small amount of material, insufficient for chemical identification, settled out. The *alcohol-insoluble* portion was fused with 8 times its weight of KOH and a portion of an aqueous extract of the fusion mixture extracted with ether. After removal of this solvent a residue was obtained, the odor of which was suggestive of formic and butyric acids. Tests (19) for formic acid confirmed its presence. No attempt was made to verify the presence of butyric acid. A number of color reactions (20), indicative of catechol, were obtained for another portion of the residue dissolved in distilled water. A small amount of the residue suspected of containing catechol was brominated in chloroform and a compound obtained which after purification melted at 189° C. Tetrabromocatechol (21) is said to melt at 192–193° C. All attempts to isolate catechol itself were unsuccessful. This concludes the investigation of the ether-soluble petroleum ether-insoluble fraction of the crude drug.

The ether-insoluble portion of the *original crude drug* was extracted with alcohol. The alcoholic extract was concentrated to a small volume, added to distilled water and filtered. Color tests made on this filtrate disclosed that tannins were probably present. Precipitation with basic lead acetate and removal of the precipitate thus formed next was undertaken. The lead-free, acid filtrate was extracted with petroleum ether, benzene and chloroform in the order named. These volatile solvents were removed in each case by distillation under reduced pressure. The respective residues were taken up in water. Portions of these aqueous solutions were tested with Fehling's and Molisch's reagents. Each of these tests was negative in every case. The remainders of the respective aqueous solutions were boiled with HCl for six hours. After neutralization, the various solutions failed to give either a positive Molisch or Fehling's test. Glucosides may be assumed to be absent from any of the extracts made by the various volatile solvents. The lead-free, acid solution was then made alkaline with NH_4OH and extracted with chloroform in the usual manner for the detection of alkaloids. The residue obtained by evaporation of the chloroform failed to react with either Wagner's or Mayer's reagent. This indicates the absence of alkaloids.

Another portion of the alcoholic extract of the crude drug was concentrated to a solid and divided into two parts. One part of the solid extract was mixed with a small amount of glycerol and heated at 190–200° C. for 20 minutes. After cooling, the glycerol mixture was diluted with water, acidified and extracted with ether. The ether was removed and the residue so obtained taken up in a small amount of water. No satisfactory evidence for the presence of phenolic compounds could be obtained from this solution although one or two qualitative tests suggested the presence of pyrogallol. The other part of the solid alcohol extract failed to reduce Fehling's solution. This extract then was heated with 10 per cent HCl on a boiling water-bath for two hours, allowed to cool, filtered and the filtrate extracted with ethyl ether. The residue obtained by evaporating the ether gave color reactions (22) said to be indicative of gallic acid. The acid-hydrolysis mixture after extraction with ether as just described was made slightly alkaline with KOH and basic lead acetate added. After filtration the filtrate was acidified and the lead removed by precipitation with H_2S. After filtration the excess H_2S was removed by aeration. This last filtrate gave a positive Molisch test and reduced Fehling's solution. A phenylhydrazine derivative was prepared from the filtrate. This derivative after purification had a melting point of 203° C. Mulliken (23) records the melting point of glucosazone (fructosazone) at 204–205° C. Another portion of the solid alcohol extract was hydrolyzed and Seliwanoff's test applied. This test was negative. The optical rotation of the solution before hydrolysis was $[\alpha]_D^{20} + 46.3$. It appears that the compound is glucose resulting from the hydrolysis of a more complex substance. The identification of glucose and gallic acid in the alcohol-soluble portion of the drug suggests the presence of a glucosidic tannin.

As recorded earlier in this paper two investigators (8), (10) have claimed that passiflora contains physiologically active material. DeNito (10) found that intravenous injection of fluid-extracts of certain species of passiflora produced a fall in blood pressure and stimulated the respiratory center of anesthetized dogs. He found, however, that oral administration of the drug failed to produce any observable effects.

In order to ascertain whether any of the various extracts of the plant contained physiologically active material a series of bio-assays was made. The ethyl ether, the petroleum ether and the alcohol-soluble constituents of the crude drug were examined. The volatile solvents were removed by distillation under reduced pressure and in each case three Gm. of the residue were suspended in 20.0 cc. of 1.0 per cent acacia solution. Carotid blood pressure and respiratory tracings were recorded for dogs under ether anesthesia. All injections were made into the femoral vein. The alcohol-soluble residue produced a sharp transient fall in blood pressure but the other residues were physiologically inert when injected.

Due to the fact that all available material was exhausted another alcoholic extraction of the crude drug was made. The solvent was removed by distillation under reduced pressure and the residue obtained extracted with water. This material was only partially soluble in the distilled water. After filtration the filtrate and residues were assayed separately. The injection of 5.0 cc. of the filtrate into a dog produced a decided fall in blood pressure. A suspension of the water-insoluble residue was made in 1.0 per cent acacia solution. When 5.0 cc. of this suspension were injected no change in blood pressure could be detected. The water-soluble portion was treated with a slight excess of 20 per cent aqueous solution of lead acetate, the solution filtered and the precipitate decomposed with sodium carbonate; after filtration the lead-free filtrate from this decomposition mixture when injected into anesthetized dogs had no effect on blood pressure. The *filtrate from the lead acetate precipitation* was next examined. The excess lead remaining in the filtrate was removed by precipitation with H_2S and the excess H_2S removed by æration. Intravenous injection of this lead-free filtrate into anesthetized dogs produced a fall in blood pressure. A portion of the filtrate was made acid and extracted successively with benzene, ethyl ether, chloroform and amyl alcohol. Another portion of the filtrate was made alkaline and extracted with a fresh portion of the above-named solvents. The volatile solvents from both the acid and alkaline extractions of the filtrate were evaporated and the residue in each case taken up in distilled water. Distilled water was used because experiments already described showed the active principle to be water soluble. No change in blood pressure was observed when aqueous extracts of the residues from the volatile solvents were injected. The aqueous solution, which had been extracted by the volatile solvents, however, still contained depressor material.

The fact that the active principle could not be removed from an acid or an alkaline solution by means of immiscible solvents would seem to indicate the absence of alkaloids, although DeNito expressed the opinion that an alkaloid was probably present. In the present experiments *with the anesthetic used* no change in respiratory rate or amplitude was observed after injection of the physiologically active material. Oral administration to non-anesthetized dogs of relatively large quantities of the aqueous extract containing the active principle failed to produce any observable effects. Further work on the pharmacology of passiflora is in progress and will be reported later.

SUMMARY.

1. The chemical constituents of *Passiflora incarnata* were separated by means of differential solvents.

2. In the lipid fraction the following substances were identified and their analytical constants recorded, myristic, palmitic, oleic, linoleic and linolenic acids; melissyl alcohol and sitosterol.

3. A small quantity of a hydrocarbon was isolated from the petroleum-ether fraction.

4. Other organic constituents identified were catechol, gallic acid and glucose.

5. The catechol was probably derived from a resin while the presence of glucose and gallic acid probably indicates that a glucosidic tannin was present in the crude drug.

6. The plant contained water-soluble depressor material.

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A NOTE ON THE VOLATILE OIL OF ILLICIUM PARVIFLORUM MICHX.*

BY P. A. FOOTE.[1]

The phytochemist in Florida has at his door many plants which should be investigated. If he fails to look for them the chances are that some will be brought to him. The writer has five new volatile oils awaiting investigation. These were brought to him by a farmer looking for commercial possibilities. Several years ago our attention was called to the large amount of oil in the leaves of *Illicium parviflorum Michx.* (Fam. *Magnoliaceæ*). This is a shrub 1–2 m. tall and according to Small (1) the habitat is in low woods and swamps in the coastal plain from Florida to Georgia. The appearance of tiny oil cells on the under side of the leaves is very pronounced. When crushed a strong sassafras odor is obtained. This is due to the high safrol content which we found to be higher than in any other oil yielding this compound. A search of the literature indicated that this oil has never been reported on.

EXPERIMENTAL.

The leaves were collected in Gainesville in October 1933. Upon steam distillation, 2.6 Kg. gave 12.1 Gm. of a colorless oil heavier than water. It stood in diffused light for three years, during which time it turned slightly yellow. A determination of the acid and ester values indi-

* Scientific Section, A. PH. A., New York meeting, 1937.

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cated but traces of them. Due to the small amount of the oil on hand and its pronounced sassafras odor it was decided to forego the determination of any more chemical and physical constants and utilize the entire amount for the determination of the chief constituent.

Separation of Safrol.—8.7 Gm. of the oil were chilled in a mixture of ice and salt to −11° C. No solidification took place even though the melting point of safrol is +8° C. Neither would a commercial sample solidify at this temperature which bore out statements in the literature that a temperature of −12° C. is necessary to solidify safrol. The oil was transferred to a centrifuge test-tube and frozen by dry ice. It was then placed in an ice-bath at 0° C. and kept there for one hour, after which it was briefly centrifuged at 1500 r. p. m. The separated liquid was poured off. It amounted to but five drops and had an odor remindful of nutmeg. An effort to prepare a solid bromine derivative of it failed.

Identification of Safrol.—The tube of crystals was immersed in a water-bath held at 11° C. They slowly melted. To determine the melting point a corrected Anschutz thermometer was immersed in the liquid and frozen there by dry ice. The tube was transferred to a water-bath held at 13° C. and the melting point observed to be 7–8° C. This was checked twice. The melting point of safrol was recorded by Eykman (2) as being 8° C. Refractive index at 20° C. was found to be 1.5340. That reported by Schimmel and Co. (3) for safrol is 1.5377 at 20° C. Using a 2.5 cc. weighing tube $d_{\frac{15}{15}}$ was 1.089 as compared to Eykman (2) 1.0960. Safrol was further identified by oxidation with an alkaline potassium permanganate solution which gave the piperonylic acid described by Fittig and Mielch (4). This oxidation must be carefully controlled because of the variety of products obtainable. Repeated trials were first made on commercial safrol. The following procedure was then adopted. Disperse 4 cc. of the sample in 240 cc. of a 1% sodium hydroxide solution contained in an 800-cc. flask. To this is slowly added with agitation 200 cc. of 5% potassium permanganate solution. Heat on a water-bath for one hour. Filter hot and cool. Acidify the filtrate with sulfuric acid. The precipitated piperonylic acid is filtered off, washed with water and recrystallized from hot alcohol. It melts at 228° C.

SUMMARY.

1. The volatile oil of *Illicium parviflorum Michx.* is largely safrol estimated at more than 90%.

2. This oil has the highest safrol content of any volatile oil yet reported.

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NOTES ON THE STABILITIES OF ATROPINE AND HYOSCYAMINE IN SOLUTION.*

BY H. H. FRICKE[1] AND K. L. KAUFMAN.[2]

The variable amounts of alkaloids obtained in the assays of the Solanaceæ drugs have been the cause of much study of the various processes. Several factors may explain the variations in the amount of alkaloids isolated. One may be the instability of atropine and hyoscyamine when heated in the various solvents. Another factor is the presence of amines and ammonia in the drugs.

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* Presented before the Scientific Section, A. PH. A., New York meeting, 1937.

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Dietzel, Schlemmer and Fischer (1) found that aqueous solutions of scopolamine, hyoscyamine and atropine showed definite changes in spectra after one hour of steam sterilization. The changes were attributed to the formation of tropic acid. However, Bjerregarrd and Schou (2) found that solutions of atropine may be sterilized 20 minutes at 120° C. without appreciable decomposition. This was not true, however, if a buffer of p_H 6.0 or higher was added.

Schaller and Baldinger (3) reported that assays completed by using a vacuum desiccator instead of a water-bath to evaporate the volatile solvents gave much higher results. DeKay and Jordan (4) found that the alkaloids of hyoscyamus were very stable and underwent no change during the assay process, that they could be heated two hours without danger of decomposition and that no hydrochlorides were formed when chloroform solutions containing them were evaporated to dryness.

Watkins and Palkins (5) found that certain brands of chloroform reacted with atropine and hyoscyamine to a considerable extent. Scoville (6) also has mentioned this tendency. As a remedy, it has been suggested to add a few cc. of dehydrated alcohol before the last few cc. of chloroform have been evaporated.

Ewe (7) felt that no notice was taken of the hydrolyzing effect upon the hyoscyamus alkaloids of the moisture which may be present in the solution. He found this must be considered and the amines must be volatilized, but that the temperature must be closely watched. He suggested removing the last two cc. of chloroform at 40° C. with a current of dried air.

EXPERIMENTAL.

The following experiments were made to determine what changes pure hyoscyamine and atropine undergo in solutions of chloroform or ether. Not less than four titrations were made in determining each average.

Six-tenths (0.6000) gram of the alkaloid was dissolved in 500 cc. of solvent. Twenty-five-cc. portions of this were evaporated on a water-bath and heated for various periods. The treatments and results are summarized in the following table.

TABLE I.

Alkaloid.	Solvent.	Quality of Solvent.	Water-Bath Treatment.	Aver. Per Cent Sample Returned.*
Atropine	Ether	U. S. P.	Evap. to dryness	100.00
"	"	"	Heated 30 min. after evap'n.	95.80
"	"	"	Heated 60 min. after evap'n.	95.23
Hyoscyamine	"	"	Evap. to dryness	100.00
"	"	"	Heated 30 min. after evap'n.	99.60
"	"	"	Heated 60 min. after evap'n.	98.86
"	"	"	Heated 120 min. after evap'n.	97.56
Atropine	"	U. S. P. Dry**	Evap. to dryness	100.00
"	"	" "	Heated 30 min. after evap'n.	95.80
"	"	" "	Heated 60 min. after evap'n.	95.30
Hyoscyamine	"	" "	Evap. to dryness	100.00
"	"	" "	Heated 30 min. after evap'n.	99.68
"	"	" "	Heated 60 min. after evap'n.	98.86
"	"	" "	Heated 120 min. after evap'n.	97.92
Atropine	Chloroform	Tech.	Evap. to dryness	87.40
"	"	"	Heated 30 min. after evap'n.	80.06
"	"	"	Heated 60 min. after evap'n.	77.10

TABLE I.—(*Continued from page 575*).

Hyoscyamine	"	"		Evap. to dryness	93.04
"	"	"		Heated 30 min. after evap'n.	92.29
"	"	"		Heated 60 min. after evap'n.	87.60
"	"	"		Heated 120 min. after evap'n.	83.45
Atropine	"	"	Dry**	Evap. to dryness	93.01
"	"	"	"	Heated 30 min. after evap'n.	93.00
"	"	"	"	Heated 180 min. after evap'n.	83.09
Hyoscyamine	"	"	"	Evap. to dryness	97.39
"	"	"	"	Heated 30 min. after evap'n.	94.38
"	"	"	"	Heated 60 min. after evap'n.	91.92
"	"	"	"	Heated 120 min. after evap'n.	90.22

* Calculations based on largest amount returned (0.03127 Gm.) as 100%.

** Dried over calcium chloride.

SUMMARY AND CONCLUSIONS.

1. Hyoscyamine and atropine are more stable in ether than in chloroform solutions.

2. Continued heating of these alkaloids on a water-bath causes their partial disappearance. This confirms the work of Schaller and Baldinger, Ewe and Scoville.

3. The results obtained show that heat is definitely a factor in the disappearance of the alkaloids from their ether solutions.

4. When chloroform is used as the solvent, some other form of destruction must also occur, as the dried chloroform gives higher results than the U. S. P. grade.

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DIAZO COLOR REACTIONS.*

BY KIRBY E. JACKSON AND WILLIAM M. DEHN.

This reaction, commonly named Ehrlich's diazo reaction, has been used to detect bile pigments in blood or in urine; also, ureoresin, urochromogen (1) and an unknown compound found in urine in cases of typhoid and measles (2). It is known that administered alcohol (3), phenols and opium bases (4) and other drugs (5) give positive Ehrlich's test with urine. It seemed important to investigate a range of other medicinals to ascertain possible fallacies when this test is applied for clinical purposes.

* From the Chemistry Laboratory, University of Washington.

The compounds to be tested, in mg. quantities, were placed in small beakers with 5 cc. of water, a few drops of acetic acid and small squares of silk cloth. A few drops of 10% sodium *nitrite* solution were added and the developed colors were read. Next ammonia was added to make alkaline solutions and these mixtures were boiled for three minutes; the developed colors were read. Finally the silk was removed, washed with water and pasted on cardboard, so that these colors could be read and preserved. The colors developed on silk gave variety and permanent records for comparisons.

In the paragraphs of the following table, the first reading is the color in acetic acid solution; the second, in ammoniacal solution; the third, on silk.

TABLE.

Colorless. Colorless. Colorless. Acetanilid, aconitine, agaricin, allonal, alypin, apothesine, aspirin, stophan, atropine, barbital, beta-eucaine, caffeine, chloral hydrate, cinchonine, cinchophen, cumarin, cuminol, exalgin, gelseminine, geraniol, homatropine, neocinchophen, numoquin, alpha-picoline, phenacetin, quinine, saccharine (6), stovaine, sugars (simple), strychnine, sulfonal, trional, tropocaine, urotropin.

Colorless. Colorless. Ivory or buff. Amygdalin, cadaverine, convallarin, elaterin, holocaine, hyoscyamine, papain, phenolphthalein, picrotoxin, quinaldine, salol, santonin, sapotoxin.

Colorless. Yellow. Ivory or buff. Anisole, convallamarin, digitalin, podophyllotoxin, salicin, saponin, salicylic acid, tannic acid, vanillin, veratrole.

Colorless. Yellow or orange. Yellow or yellow orange. Anesthesin, butyn, ethyl acetoacetate, procaine, tutocaine. *Colorless. Yellow. Colorless.* Isatin, thymol (7). *Colorless. Red. Orange-red.* Alpha-naphthylamine. *Colorless. Orange. Light brown.* Phenylhydrazine.

Yellow or orange. Yellow or orange. Buff, yellow, brown, orange. Aniline, *o*-anisidine, apomorphine, arbutin, benzidine, catechol, colchicine, *m*-cresol, *o*-cresol, *p*-cresol, eugenol, guaiacol alpha-naphthol, beta-naphthol (8), nicotine, nux vomica, phenetole, phenol (9), phloridzin (10), pyridine, pyrogallol, quinol, resorcinal (7). *Brown. Brown. Brown.* Beechwood creosote, phlorglucinol. *Orange. Green. Green.* Methylethylaniline, propylaniline. *Yellow. Red. Gray-ivory, orange or red.* Gallic acid, morphine, beta-naphthylamine. *Blue. Blue. Orange-yellow.* Antipyrine (11). *Blue. Blue. Black.* Methylargyl. *Green. Yellow. Buff.* Strophanthin. *Pink. Red. Violet-red.* Aloin. *Brown. Green. Green.* Dimethylaniline. *Red. Orange. Gray-ivory.* Diphenylhydrazine. *Orange. Brown. Gray-ivory.* Diresorcinol. *Colorless. Rose Buff.* Isoquinoline.

SUMMARY.

Diazo color reactions are very useful for identifying many compounds. Its use, for a single compound, may lead to fallacy, because many other compounds may give the same colors. Its use for detection of pathological components of urine is rendered unreliable when certain drugs have been administered. The use of silk to fix colors renders the use of the diazo reaction more accurate because the color can be kept as a permanent record.

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METHENAMINE AS A QUALITATIVE REAGENT.*

BY KIRBY E. JACKSON AND WILLIAM M. DEHN.

The use of methenamine in sulfuric acid for color reactions has been limited to tests for hordenin (1), opium alkaloids (2) and antipyrin (3). Because it yields brilliant colors with these compounds, it was thought to be of interest to test its applicability and reliability in connection with other compounds. This was done and, whereas the list of compounds giving colors with the reagent can easily be extended, enough were tested to show the formation of a variety of colors and to show the possibility of confusion of one compound with another, especially in toxicological analyses. For example, each color given by the several alkaloids is duplicated by some totally unrelated compound. Therefore, color reactions, if limited to a single test for the purpose of identification, may lead to error. However, when the material is carried through the proper methods of separation, then color tests are of great value, especially when identity is confirmed by other chemical and physiological properties.

Since methenamine is used in medicine and in certain embalming fluids, or it may be formed in the cadaver from embalming fluids containing formaldehyde or trioxymethylene and ammonia resulting from putrefaction, it is obvious that materials separated in toxicological analysis and treated with concentrated sulfuric acid can give apparent tests for alkaloids (4).

In the following tests we have used a more diluted reagent than recommended by the earlier investigators. We dissolved 0.1 Gm. in 80 cc. concentrated sulfuric acid in the cold. To a few mg. of the substance to be tested and contained in a vial, 2–3 cc. of the reagent were added. With many compounds colors are produced immediately; many colors deepen on standing. Since time, concentration and temperature can vary the colors, the shade of color reported may vary with repetition of the test. For certainty of identity of unknowns, a check on pure suspected compounds should be made.

ALKALOIDS AND OTHER COMPOUNDS.[1]

The colors observed on using the indicated reagent are described as follows:

Colorless: brucine, caffeine, cocaine, coniine, duboisine, physostigmine, pilocarpine, sparteine, strychnine, theobromine, tropacocaine (alypin, anesthesin, antipyrin, barbitol, beta-eucaine, butyn, cadaverine, cumarin, exalgine, holocain, procaine, stovaine, sulfonal, trional, tutocaine, uric acid). *Colorless, yellow:* aconitine, atropine, cinchonine, cinchonidine, daturine,

* From the Chemistry Laboratory, University of Washington.

[1] Compounds in parenthesis are non-alkaloidal compounds matching the colors of the indicated alkaloids. The use of the comma between colors means *changing to*.

hyoscine, quinidine, quinine (allonal, acetanilide, 2,4-dinitrophenol). *Colorless, red or violet:* gelseminine, hydrastine, hyoscyamine, narcotine (phenacetin). *Yellow:* cephaeline, hydrastine, nicotine, stypticin (apothan, cinchophen, edinol, numoquin, papain). *Yellow, orange or red:* agaricine, colchicine, emetine, pelleterine, solanine (*o*-nitrophenol, orcinol, phlorglucinol, resorcinol). *Colorless or yellow, dark brownish red or black:* apocodeine, aspidospermine, berberine, cannabine, chelidonine, codeine, delphine, dionin, heroin, homatropine, narceine, sanguinarine (agaricin, capsicin, elasterin, podophyllotoxin, santonin, styptol, thiophenol, tyrosine). *Purple, black:* apomorphine. *Blue, purplish red:* boldine. *Purple, indigo:* morphine. *Yellow, deep purple:* veratrine. *Rose, black:* yohimbine.

OTHER MEDICINALS.

Green darkening on standing: methargyl. *Pink, red, brownish red:* apothesine. *Violet, purplish black:* eikonogen. *Pink, carmine:* aspirin. *Orange red, carmine:* phenolphthalein. *Yellowish green, dark greenish yellow, finally olive-green:* gallic acid. *Greenish yellow, red brown:* vanillin. *Permanganate red:* veratrole. *Brownish red, purplish black:* guaiacol. *Brownish yellow deepening on standing:* thymol. *Cherry-red, deepening on standing:* carvacrol, salol. *Deep purplish red:* catechol. Cherry-red: phenol, phenetole. *Deep cherry-red:* *o*-cresol, *m*-cresol, eugenol. *Violet brown, deep purplish blue:* beechwood creosote. *Brownish red:* anisole, *p*-cresol, diresorcinol, pyrogallol.

PHENOLS, THEIR DERIVATIVES AND OTHER COMPOUNDS.

Colorless, developing turbidity: *p*-hydroxybenzoic acid. *Greenish yellow, yellow:* 3,5-dinitro-*o*-cresol, 8-hydroxyquinoline. *Turbid light pink, cloudy violet:* 2,4-dichlorophenol. *Light green, greenish yellow:* *m*-hydroxybenzoic acid. *Carmine, pink, colorless, turbid:* *o*-hydroxybenzoic acid. *Grass green, dark olive green:* 2-hydroxy-3-naphthoic acid. *Brownish black:* quinol, alpha-naphthol. *Deep reddish brown:* tricresol, isoeugenol, nitroso-beta-naphthol, apiol. *Deep brownish red:* *o*-cresylacetate, eugenolmethylether. *Reddish brown:* alizarin, cuminol. *Violet black:* *o*-hydroxydiphenyl. *Greenish black:* beta-naphthol.

SUMMARY.

Manseau's reagent is very useful in testing for alkaloids, medicinals and other compounds. For toxicological purposes its use may lead to fallacies. Many closely related compounds, for example, the hydroxybenzoic acids, the cresols, etc., can be differentiated.

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(3) Mannich, *Arch. Pharm.*, 250, 647 (1912); *Apoth. Ztg.*, 27, 535 (1912). Antipyrin gives a white precipitate.

(4) For a court case in which strychnine and codeine with embalming fluid were confused, see *J. Chem. Education*, 7, 559 (1930).

CHRISTOPHER KILMER MARRIES.

Christopher Kilmer, son of Joyce Kilmer, the poet, famous as the author of "Trees," and grandson of Dr. Frederick B. Kilmer, member of the A. PH. A., was married on July 29th to Miss Alberta Taylor Daniel, of Arlington County, Virginia.

VOLATILE OIL IN SANDALWOOD.*

BY J. F. CLEVENGER.[1]

Sandalwood is usually imported from India. Non-official species in limited amounts have also been imported from Australia, New Caledonia Islands and Africa.

During the past six years, most of the importations of sandalwood in New York have been analyzed for the yield of volatile oil. Determinations have also been made of some of the physical and chemical characteristics of these oils.

The results here reported were obtained by the method outlined in the Methods of Analysis A. O. A. C., 4th Edition (1935), 447–449. Santalol content was determined by the method outlined in U. S. P. XI, page 267, for sandalwood oils, except that the amount of oil usually available (approx. 5 cc.) was used instead of 10 cc. as indicated in the method.

It is of interest to note that Sandalwood requires a prolonged period for distillation—approximately twenty-four hours. This period is only a fraction of that used commercially (approximately 5 days). It may be pointed out that only the last fractions of volatile oil obtained are subjected to this prolonged period of heating.

The moisture was determined by xylol distillation.

A tabulation of representative results of the determinations made follows:

Authentic Sandalwood.

Yield V/W.*	Moist.	Sp. Gr. 25°/25° C.	Op. Rot. 25° C.**	Ref. Ind. 20° C.	Santalol.
8.0	7.2	0.971	−19.0	1.505	96
5.0	6.2	0.973	−17.3	1.504	92
4.0	6.3	0.964	−18.3	1.504	93
4.0	7.5	0.968	−16.9	1.503	89
4.0	6.0	0.976	−16.2	1.503	90
7.0	6.3	0.964	−22.5	1.503	96
4.3	4.0	0.973	−16.6	1.504	94
8.2	7.3	0.974	−20.4	1.504	96
6.0	7.0	0.982	−19.7	1.507	96
6.0	7.5	0.982	−20.5	1.508	96
5.2	8.4	0.984	−18.7	1.507	94
6.5	6.4	0.976	−22.1	1.506	97
4.0	6.5	0.985	−17.3	1.506	96
4.5	5.0	0.978	−21.7	1.503	95
4.7	9.1	0.970	−19.4	1.507	97
3.5	...	0.979	−22.5	1.505	90
3.0	...	0.982	−15.9	1.506	96

Sandalwood Chips.

Yield V/W.*	Moist.	Sp. Gr. 25°/25° C.	Op. Rot. 25° C.**	Ref. Ind. 20° C.	Santalol.
1.5	4.0	0.973	−19.0	1.502	92
1.4	...	0.973	−18.0	1.503	96
3.0	...	0.973	−15.2	1.503	..
3.5	7.4	0.974	−18.0	1.505	..

* Presented before Scientific Section, A. PH. A., New York meeting, 1937.

[1] U. S. Food and Drug Administration, New York, N. Y.

2.0	...	0.982	−19.4	1.507	96
3.2	...	0.978	−18.0	1.506	96
2.0	...	0.978	−20.0	1.505	..
0.8	...	0.982	−20.8	1.507	..
		Non-Official Varieties of Sandalwood.			
2.4[1]	8.4	0.983	−12.4	1.504	96
5.0[1]	8.8	0.960	− 9.3	1.509	94
3.5[1]	5.0	0.983	−13.2	1.504	96
1.2[2]	...	0.954	0.0	1.505	..
2.0[2]	...	0.954	− 9.7	1.504	..
5.0[2]	...	0.978	−14.5	1.504	96
6.0[2]	...	0.975	−14.4	1.504	..
2.5[3]	...	0.954	−38.0	1.507	87
2.0[4]	...	0.943	+12.1	1.500	49

* Cc. per 100 Gm. of sandalwood.

** Reported in angular degrees on the basis of a 100-mm. tube.

[1] Australian Sandalwood.

[2] From New Caledonia Islands.

[3] African Sandalwood.

[4] Amyris Species.

CONCLUSIONS.

1. The yield of volatile oil in authentic sandalwood was found to vary considerably, being least in sandalwood chips, the amount in some instances falling below one per cent. This low yield in volatile oil in the chips is probably due, for the most part, to the inclusion of a larger proportion of sap wood.

2. The specific gravity of the volatile oil is sometimes outside the Pharmacopœial limits (0.965 to 0.980 at 25° C.), values both above and below the specifications being noted. The optical rotation in the case of some specimens falls outside the official limits (−15° to −20° in a 100-mm. tube at 25° C.), value as high as −22.50 being observed. The refractive index for the volatile oils from both the official and non-official varieties of sandalwood are essentially identical, the entire range lying between 1.500 and 1.509.

3. The specific gravity and refractive index are of little value for the detection of non-official varieties of sandalwood. The optical rotation, on the other hand, is particularly important. In the case of the volatile oils from the nine non-official samples reported not one possessed the optical rotation within the official limits, or within the limits of the volatile oils from the authentic sandalwoods.

THE HYDROLYSIS OF MENTHYL ACETATE AND ACETYLIZED PEPPERMINT OIL. II.*

BY LAWRENCE H. BALDINGER.[1]

INTRODUCTION.

In the analysis of peppermint oil the United States Pharmacopœia directs that 50 cc. of half-normal alcoholic potassium hydroxide be used to hydrolyze the weighed sample of acetylized oil. Provided an excess of base is assured, it should

* Scientific Section, A. PH. A., New York meeting, 1937.

[1] Department of Pharmacy, University of Notre Dame, Notre Dame, Indiana.

be possible to use a smaller quantity of the hydroxide solution for the hydrolysis. In order to test the validity of this assumption and to supplement semi-quantitatively the determination of the reaction velocities between menthyl acetate and acetylized peppermint oil and potassium hydroxide the following experiments were performed:

Accurately weighed 5-cc. samples of acetylized peppermint oil were treated with alcoholic potassium hydroxide solution, one with 30 cc., the next with 35 cc., up to 50 cc. The flasks were placed in a water-bath, thermostatically controlled to a desired temperature within 0.2° C. The contents of the flasks, after being kept at the desired temperature for one hour, were titrated with half-normal sulfuric acid immediately after being taken from the bath. Phenolphthalein was used as the indicator. From the data obtained the percentage of menthol in each 5-cc. portion was calculated using the formula of the U. S. P. monograph for oil of peppermint. Assuming that the hydrolysis of the acetylized oil was complete at steam-bath temperatures, the percentage of menthol obtained in a series of experiments at that temperature was used as a basis upon which to calculate the per cent hydrolysis taking place at the different temperatures which were used. See Tables I and II.

From these results it may be roughly concluded that steam-bath temperature is necessary for complete hydrolysis of the oil and that a volume of alcoholic potassium hydroxide less than 50 cc. may give erratic results. The lower percentages obtained when using smaller portions of potassium hydroxide may be due to the reading error involved in the back titration of the excess alkali with acid.

TABLE I.—PER CENT OF MENTHOL IN PEPPERMINT OIL.

	Cubic Centimeters of Alcoholic Potassium Hydroxide Solution Originally Added to Samples of Acetylized Peppermint Oil.				
Temperature.	30.	35.	40.	45.	50.
25° C.	20.96	22.22	22.36	23.43	23.82
30° C.	27.76	32.85	31.62	28.67	29.06
35° C.	30.34	32.03	33.56	33.59	34.46
40° C.	34.39	38.98	39.16	40.30	40.50
45° C.	34.99	36.39	40.17	42.11	42.86
100° C. Steam-bath	47.60	48.57	48.51	49.26	49.31

TABLE II.—PERCENTAGE HYDROLYSIS OF ACETYLIZED PEPPERMINT OIL.

	Cubic Centimeters of Alcoholic Potassium Hydroxide Solution Originally Added to Samples of Acetylized Peppermint Oil.				
Temperature.	30.	35.	40.	45.	45.
25° C.	43.07	45.65	45.94	48.14	48.94
30° C.	57.04	67.50	64.97	58.91	59.71
35° C.	62.34	65.81	68.95	69.02	70.80
40° C.	70.66	80.09	80.46	82.80	83.21
45° C.	71.89	74.46	82.54	86.52	88.06

In a previous publication (1) the reaction rates of the hydrolysis of acetylized peppermint oil and of pure menthyl acetate at 25° C., 30° C. and 35° C. were reported. This paper is a report of further work on the problem using temperatures of 40° C., 45° C. and 50° C.

REAGENTS AND EXPERIMENTAL DATA.

The peppermint oil used in this work had the following constants: Optical rotation, $-21.8^{27°}_{D}$; esters, 9.46%; total menthol, 51.65%. The other reagents,

the experimental procedure and the theoretical discussion are the same as reported previously (*loc. cit.*). See Tables III and IV.

TABLE III.—HYDROLYSIS OF MENTHYL ACETATE.

	40° C.		45° C.		50° C.	
t (Minutes).	$k \cdot 10^2$.	% Hydrolysis.	$k \cdot 10^2$.	% Hydrolysis.	$k \cdot 10^2$.	% Hydrolysis.
0						
15	8.63	25.3	11.82	33.3	18.65	44.4
30	9.59	43.8	12.58	52.8	18.89	63.6
45	9.33	54.2	10.61	59.3	18.13	73.0
60	9.50	62.5	11.25	68.5	19.42	81.0
75	10.31	70.5	11.95	75.6	17.95	83.9
90	9.69	73.4	9.27	75.9	19.91	88.8
105	10.06	77.9	12.03	82.8	18.31	89.8
120	10.07	80.8	13.63	87.5	19.30	92.4
135	10.53	84.0	12.92	88.6	19.02	93.6
150	10.96	86.8	12.58	89.7	21.85	95.9
170	10.94	88.6	12.30	91.2	20.27	96.3
190	10.40	89.5	12.78	93.0	20.65	97.2
210	10.36	92.1	13.93	95.0	20.41	97.3
240	...	..	13.32	95.8	19.81	98.2
270	...	..	12.85	96.4	...	..
	Av. 10.1		Av. 12.47		Av. 19.47	

TABLE IV.—HYDROLYSIS OF ACETYLIZED PEPPERMINT OIL.

	40° C.		45° C.		50° C.	
(Minutes).	$k \cdot 10^2$.	% Hydrolysis.	$k \cdot 10^2$.	% Hydrolysis.	$k \cdot 10^2$.	% Hydrolysis.
0	..	..	...	..	...	..
10	..	..	...	..	18.78	36.5
15	..	..	11.07	30.8	...	..
20	..	..	...	..	17.28	54.5
30	..	..	11.92	52.4	16.10	65.1
40	..	..	...	..	16.28	74.4
45	8.93	57.3	11.95	65.4	...	..
50	..	..	...	..	15.63	79.6
60	8.62	65.4	12.75	76.2	16.11	85.1
75	9.23	74.6	11.03	78.5	16.84	90.8
90	8.77	78.2	9.89	80.5	14.04	90.8
105	8.19	80.5	9.70	83.9	...	..
120	8.38	84.6	...	..	14.09	95.36
135	8.41	87.4	...	..	...	..
150	8.58	90.1	9.77	91.8	...	..
170	7.79	90.5	11.11	95.5	...	..
190	7.56	92.0	10.15	95.5	...	..
230	..	..	9.53	97.0	...	..
250	7.89	96.3	10.77	98.5	...	..
270	..	..	9.97	98.5	...	..
	Av. 8.40		Av. 10.5		Av. 16.28	

DISCUSSION.

As a result of a large number of observations on a variety of chemical reactions it has been shown that in general the velocity of a reaction is doubled or trebled for an increase in temperature of ten degrees. From the data given in Table V the reaction-constant ratios over ten-degree changes have been calculated and are as

follows: Menthyl acetate, k_{35}/k_{25} (obs.) 2.25; k_{40}/k_{30} (obs.) 2.34, (calc.) 2.19; k_{45}/k_{35} (obs.) 2.16, (calc.) 2.14; k_{50}/k_{40} (obs.) 1.93, (calc.) 2.09: Acetylized Peppermint Oil, k_{35}/k_{25} (obs.) 2.34; k_{40}/k_{30} (obs.) 2.06, (calc.) 2.28; k_{45}/k_{35} (obs.) 1.87, (calc.) 2.22; k_{50}/k_{40} (obs.) 1.93, (calc.) 2.17. One of the several empirical expressions which have been suggested for representing the variation of the velocity constant, is that proposed by van't Hoff and verified by Arrhenius (2), $d\,lnk/dT = Q/R.\ 1/T^2$, where Q is a constant and R is the gas constant. Integration of this expression between the limits k_1, T_1 and k_2T_2 gives the expression $ln\ k_1/k_2 = Q/R\,(1/T_2 - 1/T_1)$. The Arrhenius equation may also be written $1/k.dk/dT = Q/RT^2$, where $1/k.\ dk/dT$ is the temperature coefficient of the reaction per unit velocity per degree rise in temperature; the rate of increase with temperature of reaction velocity will therefore diminish with rise in temperature. The custom is to call the ratio of the velocities at two temperatures ten degrees apart the temperature coefficient; usually 35° C. and 25° C. are selected so that k_{35}/k_{25} is usually referred to as the temperature coefficient of a reaction. For this range of temperature, the Arrhenius equation becomes $2.303 \log k_1/k_2 = Q/1.9864\ (1/308 - 1/298)$ so that $\log k_2/k_1 = Q/41{,}988$.

For the hydrolysis of menthyl acetate by potassium hydroxide the value of Q is 14,768 k_{35}/k_{25} is 2.248; for the hydrolysis of acetylized peppermint oil by potassium hydroxide, the value of Q is 15,531 and k_{35}/k_{25} is 2.344. In Table V are tabulated the reaction constants of this and previous work, also calculated values for reaction constants at those temperatures used in the experiments. The calculated values were determined using the value of Q obtained with temperature coefficient k_{35}/k_{25}.

TABLE V.—REACTION CONSTANTS, OBSERVED AND CALCULATED.

T	Menthyl Acetate, $k \cdot 10^3$.		Acetylized Peppermint Oil, $k \cdot 10^3$.	
Abs.	Obs.	Calc.	Obs.	Calc.
298	2.57	...	2.40	...
303	4.33	3.88	4.06	3.70
308	5.77	...	5.62	...
313	10.11	8.49	8.40	8.43
318	12.47	12.34	10.51	12.48
323	19.47	17.72	16.13	18.29

CONCLUSION.

While it has been observed that the velocity of the hydrolysis reaction is approximately doubled for a ten-degree rise in temperature, the lack of agreement in the results for acetylized peppermint oil would appear to indicate that either counter reactions or side reactions take place between the potassium hydroxide and constituents in the oil other than the menthyl acetate. Experimental difficulties may also account for some of the results since at the higher temperatures the reaction proceeds at a much faster rate. While the problem is chiefly of theoretical interest, it is apparent from a practical point of view that a fair excess of base is necessary in such reactions and that heating of such reaction mixtures to a fairly high temperature for at least one hour will do much to insure concordant results.

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A PHYTOCHEMICAL STUDY OF IPOMEA PES-CAPRÆ.*,[1]

BY GUSTAV E. CWALINA[2] AND GLENN L. JENKINS.[3]

Ipomea Pes-Caprœ (L.) *Sweet, Family Convolvulaceæ*, is a perennial plant with a tough woody root as thick as the finger and many feet in length; it abounds in the sand on the seashore; from the enlarged crown of the root grow a number of creeping stems, fleshy and purplish when young but becoming woody as they mature; the shape of the leaf is indicated by the name of the plant.

Detailed botanical descriptions of this plant are given by Small (1), Gray (2), Pardo de Tavera (3), Grisebach (4), Dymock (5) and Schimper (6).

According to Campbell (7), *Ipomea Pes-Caprœ* is a denizen of nearly every tropical beach the world over. Guppy (8) states that as a rule this plant monopolizes the thirtieth parallels of north and south longitude. In the United States it is described (1, 2) as growing on sandy beaches from South Carolina to Texas.

Dragendorff (9) states that the leaves are used in New South Wales against rheumatism and dropsy and also for sores and ulcers. The Indian Materia Medica (10) gives the following medicinal uses: a decoction (1 to 20) of the roots and leaves is used in rheumatism, gout, gonorrhea and dropsy in doses of one to two ounces; the plant is supposed to have the power of preventing the effects of age; the juice is given as diuretic in dropsy and the bruised leaves are applied to the parts; the paste of the leaves is applied to boils and carbuncles; the leaves boiled in water are applied locally to painful joints in rheumatism and to the abdomen in colic.

No report of any chemical investigation of this drug has been found.

EXPERIMENTAL.

Ten kilograms of the air-dried aerial portions of *Ipomea Pes-Caprœ* were received from T. K. MacDonell in Florida. The stems weighed six Kg.; the leaves and flowers, four Kg. Nine kilograms of this material in the above ratio (6:4) were ground to a fine powder in a Koerner mill. The remaining kilogram was separated into stems and leaves and flowers and ground separately. Representative portions of the stems and of the leaves and flowers were analyzed for the customary drug "constants" using the procedures of the U. S. P. X. The results are recorded below:

For the Stems:

	Per Cent.		
	A.	B.	Average.
Total Volatile at 110° C.	7.70	7.64	7.67
Petroleum Benzin-Soluble Extractive			
Volatile	0.14	0.12	0.13
Non-Volatile	4.50	3.83	4.17
Ether-Soluble Extractive			
Volatile	0.36	0.55	0.46
Non-Volatile	4.50	4.12	4.31
Alcohol-Soluble Extractive	8.67	8.56	8.62

* Presented before Scientific Section, A. PH. A., New York meeting, 1937.

[1] From the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Maryland. Abstracted from a thesis submitted by G. E. Cwalina to the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Alumni Research Scholar.

[3] Professor of Pharmaceutical Chemistry, University of Minnesota, College of Pharmacy.

Diluted Alcohol-Soluble Extractive	18.03	18.43	18.23
Water-Soluble Extractive	13.96	14.23	14.10
Total Ash	7.25	7.41	7.33
Acid-Insoluble Ash	0.05	0.07	0.06

For the Leaves and Flowers:

	Per Cent.		
	A.	B.	Average.
Total Volatile at 110° C.	8.67	8.62	8.65
Petroleum Benzin-Soluble Extractive			
Volatile	0.085	0.096	0.091
Non-Volatile	4.60	4.83	4.72
Ether-Soluble Extractive			
Volatile	0.25	0.39	0.32
Non-Volatile	5.61	6.49	6.05
Alcohol-Soluble Extractive	11.41	11.09	11.25
Diluted Alcohol-Soluble Extractive	22.94	22.31	22.63
Water-Soluble Extractive	17.32	17.88	17.60
Total Ash	14.06	14.07	14.07
Acid-Insoluble Ash	0.11	0.00	0.06

By way of preliminary examination a 49.28-Gm. portion of the mixed drug was extracted successively according to the Dragendorff scheme (11) in a Soxhlet extractor. The extraction was continued for at least ten hours in every case. The solvents were removed on the steam-bath and the residues dried in a desiccator over sulfuric acid to constant weight. The residues were then placed in an oven at 110° C. and heated until their weights again became constant.

		Per Cent.	
		Total.	Non-Volatile at 110° C.
Petroleum Benzin (b. p. 30–60° C.) extracted		3.39	3.36
Ether	"	1.14	1.00
Chloroform	"	0.59	0.54
Alcohol	"	13.51	10.73
Water	"	16.56	14.90

Tests on the petroleum-benzin extract indicated the presence of sterol, fat and volatile oil. The ether, chloroform and alcohol extracts were of a resinous nature. The alcohol extract, in addition, gave positive tannin reactions. The water extract gave no definite indications.

Tests for alkaloid by maceration with Prollius' fluid as well as with an aqueous acid solution were negative.

Examination by the Lead Method (12) revealed the presence of fat, tannin, red coloring matter and reducing carbohydrate.

Examination of 100 Gm. of the drug by the Stas-Otto Method (13) gave negative tests for alkaloids but did give positive tests for tannin.

The total resin content of the drug as determined by the method of Power and Rogerson (14) was 7.27 per cent.

The ash of the drug contained the sulfate, chloride and carbonate radicals and the following metals: tin, iron, aluminum, magnesium, calcium, sodium and potassium. Since the ash was not completely dissolved by treatment with hot dilute nitric acid, silica was probably present also.

One kilogram of drug yielded 0.48 Gm. of volatile oil corresponding to a yield of 0.048 per cent. Most of the oil distilled as a lemon-yellow liquid between 127° C. and 145° C. at 20 mm. The distilled oil had a specific gravity 28° C./28° C. of 0.9626; refractive index at 26° C., 1.4703. An alcoholic solution was neutral to litmus. The oil had an aromatic odor similar to that of the crude drug. It solidified on long standing.

LARGE SCALE EXTRACTION. PETROLEUM-BENZIN EXTRACT.

Nine kilograms of the ground drug, in a number 60 to 80 powder, were extracted by percolation with petroleum benzin (b. p. 30–60° C.). The solvent was recovered from the percolate and

used again for further percolation. Extraction was continued until the percolate came through colorless and left no appreciable residue upon evaporation of a liter of percolate. The petroleum benzin was removed as completely as possible by distillation under reduced pressure over a water-bath. The extract thus obtained weighed 352 Gm., representing 3.91 per cent of the drug. It had a wax-like appearance and consistence, a black-green color and an aromatic odor.

The extract was washed repeatedly with ethyl acetate, being thus separated into an ethyl acetate-soluble and an ethyl acetate-insoluble portion. The solution had a black-green color. The residue was a yellowish wax-like solid and weighed approximately 25 Gm.

Examination of the Ethyl Acetate-Insoluble Material.—This material was washed many times on a Büchner funnel with ethyl acetate. The ethyl acetate was removed completely from the residue and crystallization from various solvents attempted but without success.

The residue was then saponified, first, by the method of Bomer (15) using a 25 per cent solution of potassium hydroxide in 70 per cent alcohol and refluxing for two and a half hours and, secondly, by refluxing for six hours with a 12 per cent solution of potassium hydroxide in 95 per cent alcohol. This treatment yielded, after removal of the ether used, a solid unsaponifiable portion and an alkaline hydro-alcoholic solution of saponifiable material.

The Unsaponifiable Portion.—The unsaponifiable matter was boiled for 2 hours in an acetylation flask with an equal weight of acetic anhydride. At the end of boiling two layers were present in the flask. The upper yellowish oily layer was separated and washed thoroughly with boiling water. It solidified on cooling to room temperature. It would not crystallize from various organic solvents. The lower aqueous layer did not yield anything definite.

Isolation of Pentatriacontane.—The solidified upper layer was now treated by the method of Leys (16) for the separation of hydrocarbons and alcohols. It was dissolved in 100 cc. of iso-amyl alcohol, 100 cc. of concentrated hydrochloric acid were added and the mixture heated to boiling with continuous stirring. The mixture was allowed to cool slowly by immersing the beaker in warm water and allowing it to stand until it had cooled down to room temperature. The solid cake which formed at the surface was lifted off, boiled again with iso-amyl alcohol and concentrated hydrochloric acid, cooled slowly and the cake again lifted off and washed several times with hot water. The lower layers in each of the above procedures consisted of brown, gelatinous masses which on evaporation to dryness on a water-bath became black, wax-like solids. This material could not be purified and was discarded. The solid cake was dissolved in hot iso-amyl alcohol and boiled with charcoal. The solution was filtered through a steam-heated funnel. After removal of the iso-amyl alcohol the residue was dissolved in 300 cc. of ether-chloroform mixture (3:1). The solution on standing over night in a refrigerator threw down a precipitate which was filtered off with suction and washed with cold ether-chloroform mixture. The substance remaining on the filter paper was an almost white powder. It was purified by precipitation from chloroform as follows: the material was dissolved in chloroform at room temperature and placed in the refrigerator at 8° C. for several hours; the precipitate formed was filtered off and washed with cold chloroform. After several repetitions of this procedure, about 0.4 Gm. of a perfectly white powder melting constantly at 74.5° C. was obtained. A carbon tetrachloride solution of a portion of this substance did not decolorize a solution of bromine in carbon tetrachloride either in the hot or in the cold.

3.458 mg. gave 11.115 mg. CO_2 and 4.622 mg. H_2O.
C = 85.44 per cent; H = 14.58 per cent.
$C_{35}H_{72}$ requires C = 85.27 per cent; H = 14.73 per cent.

The melting point of pentatriacontane is reported in Beilstein (17) as 74.7° C.

The above-described substance from its manner of isolation, its elementary analysis and its melting point was identified as pentatriacontane.

The Saponifiable Portion.—Most of the alcohol was removed from the alkaline hydro-alcoholic solution of saponifiable material by distillation over a steam-bath. The concentrated solution was made acid with sulfuric acid (1 to 3) and shaken out with ether. The slightly green-colored ether solution was dried over anhydrous sodium sulfate and the ether removed. The residue, consisting of a few cubic centimeters of a brown liquid, was dissolved in hot 95 per cent alcohol. Upon standing in the refrigerator for a few hours the solution deposited a light brown precipitate. This was filtered off with suction, dissolved in ethyl acetate, and the solution placed in a

refrigerator over night. Several milligrams of light yellow crystals melting at 79–80° C. were obtained. On recrystallization from hot alcohol the crystals although almost white still melted at 79–80° C. The amount of the crystals was too small to permit running further tests. This substance was probably behenic acid which was obtained subsequently in sufficient quantity to be definitely characterized.

The concentrated alkaline liquid after acidification and extraction with ether was evaporated almost to dryness on a steam-bath. The residue was extracted with an ether-alcohol mixture (3 parts of 95 per cent alcohol and 1 part ether). The ether-alcohol solution was boiled twice with charcoal, filtered and evaporated to dryness. The residue was taken up in water and 12 per cent barium chloride solution added until no further precipitation occurred. The filtrate was concentrated to a thick syrup which when heated with potassium bisulfate produced the characteristic pungent choking odor of acrolein. The presence of glycerin was indicated by this test.

Examination of the Ethyl Acetate-Soluble Material.—The portion of the petroleum-benzin extract soluble in ethyl acetate amounted to 325 Gm. Of this, 150 Gm. were refluxed for 6 hours with 1500 cc. of an alcoholic solution of potassium hydroxide (300 Gm. of potassium hydroxide in 1500 cc. of 70 per cent alcohol). Most of the alcohol was distilled off, water added and the mixture extracted repeatedly with 200-cc. portions of ether. This treatment yielded an ether solution of unsaponifiable material and an aqueous alkaline solution of saponifiable material.

The Unsaponifiable Portion.—The ether solutions were combined, washed with water and the ether removed. The yellow-green residue was dissolved in 95 per cent alcohol and placed in the refrigerator over night. A yellow solid separated. This was filtered off and the filtrate concentrated slightly. Another small portion of yellow material was obtained on cooling. The filtrate from this was replaced in the refrigerator. A quantity of gleaming white platelets was slowly deposited over a period of two days. By concentration and further cooling two more fractions of white platelets were obtained. No further crystalline material could be obtained from the mother liquor from which the yellow solid and the white platelets had been obtained.

Isolation of Triacontane.—The two portions of yellow solid material totaling about 2 Gm. were combined and recrystallized several times from 95 per cent alcohol. The top fraction still had a yellow color; therefore it was recrystallized from ethyl acetate. It was obtained as an almost white precipitate melting somewhat indefinitely in the neighborhood of 65° C. It was dissolved in chloroform and the solution boiled with charcoal. On filtration and concentration it was obtained as a perfectly white fine powder melting at 64–65° C. Recrystallization from ether did not change the melting point. The substance did not give positive sterol reactions, it did not react with hot or cold concentrated sulfuric acid and it could not be acetylated. It was insoluble in 10 per cent solutions of hydrochloric acid and sodium hydroxide.

The above-described substance from its manner of isolation and its chemical inactivity was undoubtedly a hydrocarbon. The constancy of its melting point after crystallization from four different solvents indicated that it was probably triacontane, $C_{30}H_{62}$.

Beilstein (17) reports triacontane as melting at 65.2° C.

Isolation of a Sterol.—The three fractions of white platelets which had been obtained from the alcoholic filtrate from the triacontane precipitates were combined and the total (0.6 Gm.) recrystallized from hot 95 per cent alcohol. After drying over night in a vacuum-desiccator over sulfuric acid the crystals melted at 133–134° C. Under the microscope they appeared as transparent, slightly elongated, five-sided rods. They were insoluble in water, in 10 per cent hydrochloric acid and in 10 per cent sodium hydroxide solution. They were soluble in cold concentrated sulfuric acid. The sulfuric acid solution had an orange-red color and showed a heavy green fluorescence by reflected light. The substance gave positive sterol reactions with the Liebermann-Burchard and Salkowski reagents. After four recrystallizations from 95 per cent alcohol the melting point was raised to 135–136° C. Two more crystallizations from absolute alcohol gave a product (about 0.15 Gm.) which melted at 136–137° C.

3.011 mg. gave 9.295 mg. CO_2 and 3.180 mg. H_2O.
C = 84.18 per cent; H = 11.82 per cent.
2.429 mg. gave 7.508 mg. CO_2 and 2.549 mg. H_2O.
C = 84.29 per cent; H = 11.74 per cent.
Average C = 84.24 per cent; H = 11.78 per cent.
$C_{29}H_{49}OH$ requires C = 83.98 per cent; H = 12.16 per cent.

0.1106 Gm. of the sterol dissolved in 10.00 cc. of chloroform gave $\alpha_D^{28°} = -0.57°$ in a 100-mm. tube $[\alpha]_D^{28°} = -51.54°$.

A chloroform solution of the sterol decolorized a chloroformic solution of bromine.

About 0.15 Gm. of the sterol was acetylated by refluxing with 5 cc. of acetic anhydride for two hours. The acetic anhydride was removed by heating over a water-bath at 10-mm. pressure. The cream-colored crystalline residue was dissolved in chloroform and boiled with charcoal. The filtrate was evaporated to dryness and the white residue recrystallized from absolute alcohol until the crystals melted constantly at 133–134° C.

Since the saponification equivalents of the sterols are said (18) to be more reliable in the determination of the empirical formulas of sterols than combustion analyses, the acetate obtained above was saponified.

0.0793 Gm. of the acetate, dried to constant weight at 105° C., was refluxed for one hour and a half with 5.00 cc. of half-normal alcoholic potassium hydroxide solution. A blank was run simultaneously. The acetate was found to be equivalent to 1.73 cc. of 0.1 normal sulfuric acid. The molecular weight of the acetate calculated from the above was 458.4.

$$C_{29}H_{49}O.CO.CH_3 = 456.41$$

The sterol set free by the saponification was shaken out with chloroform and recrystallized three times from absolute alcohol. The melting point of the crystals rose from 133° to 137° C. The final melting point was not constant but the small amount of the sterol remaining after the third crystallization did not permit of further purification.

From its reactions with bromine, with the Liebermann-Burchard and Salkowski reagents, and with sulfuric acid; from its specific rotation; from its combustion analysis; and from the molecular weight calculated from its saponification value the above-described substance was identified as a sterol with the probable formula: $C_{29}H_{49}OH$.

The Saponifiable Portion.—The aqueous alkaline solution after extraction with ether contained the saponifiable portion of the ethyl acetate-soluble part of the petroleum-benzin extract. It was made acid with sulfuric acid (1 to 3) and extracted with ether. The ether solutions were combined, washed with water, shaken with anhydrous sodium sulfate, filtered and the ether removed. The residue had a rancid odor. It weighed approximately 85 Gm. It was dissolved in 95 per cent alcohol and placed in the refrigerator at 7° C. over night. A quantity of dark green solid material separated out. This was filtered off with suction, washed with cold 95 per cent alcohol, redissolved in hot 95 per cent alcohol and again placed in the refrigerator over night. The material which was deposited now had a light gray color. The filtrates were combined.

The above treatment produced two fractions: Fraction A, consisting of the saponifiable matter soluble in 95 per cent ethyl alcohol at 7° C. and Fraction B, consisting of the saponifiable matter precipitated from 95 per cent alcohol at 7° C.

By similar treatment of Fraction B with ether there was obtained a Fraction C, readily soluble in ether at 7° C. and a Fraction D, precipitated from ether at 7° C.

Isolation of Behenic Acid.—Fraction C, consisting of the saponifiable matter precipitated from 95 per cent alcohol at 7° but soluble in ether at 7° C., gave a red-brown residue upon removal of the ether. The residue was dissolved in hot alcohol. On cooling down to room temperature, the solution deposited a brown solid from which nothing definite could be isolated. On standing at room temperature, the filtrate from the brown precipitate deposited about 0.3 Gm. of a white solid. This was removed and recrystallized from 95 per cent alcohol until the melting point became constant at 78–79° C.

$C_{22}H_{44}O_2$ has been reported as melting at 79–80° C. (19) and at 81–82° C. (20).

0.0412 Gm. of the acid required 1.20 cc. of 0.1*N* potassium hydroxide for neutralization. The molecular weight calculated from this data is 343.3.

$C_{22}H_{44}O_2 = 340.4$.
The silver salt was prepared and analyzed.
0.0135 Gm. of silver salt gave on ignition 0.0032 Gm. of silver.
Ag = 23.70 per cent.
$C_{22}H_{43}O_2Ag$ requires Ag = 24.12 per cent.

The above-described compound was thus identified as behenic acid.

The filtrate from the behenic acid on further concentration deposited another fraction of crystals which melted at 73–74° C. Two recrystallizations from alcohol raised the melting point to 78–79° C. These crystals were evidently a further yield of behenic acid. No other material was obtained from this fraction.

Isolation of Melissic Acid.—Fraction D, consisting of the saponifiable material deposited from alcohol and from ether at 7° C., was dissolved in hot alcohol and cooled in the refrigerator. The white deposit which formed was removed and recrystallized from hot alcohol. The crystals melted at 83–84° C. Further crystallizations from alcohol gave a substance melting constantly at 87° C.

$C_{30}H_{60}O_2$ has been reported as melting at 89–90° C. (21), at 90° C. (22) and 90.6° C. (23).

0.0727 Gm. of the acid required 1.61 cc. of 0.1*N* potassium hydroxide for neutralization. The molecular weight calculated from this data is 451.6.

$C_{30}H_{60}O_2$ = 452.5.
The silver salt was prepared and analyzed.
0.0137 Gm. of silver salt gave on ignition 0.0027 Gm. of silver.
Ag = 19.71 per cent.
$C_{30}H_{59}O_2Ag$ requires Ag = 19.29 per cent.

The above-described compound was thus identified as melissic acid, the low melting point being due to the presence of a small amount of impurity.

The filtrate from the melissic acid was evaporated to dryness and the residue dissolved in hot ethyl acetate. On cooling, a few milligrams of yellowish white crystals were obtained. The filtrate was evaporated to dryness giving a light brown residue which melted at 77°. The yellow-white crystals melted at 74–75° C. The quantities of material here were so small that they could not be further worked with. The melting points of the precipitate and residue indicate that some behenic acid may have been present in this fraction as well as in Fraction C.

Fraction A consisted of the saponifiable material soluble in 95 per cent alcohol at 7° C. The alcohol was removed under reduced pressure. The residue weighing about 50 Gm. was mixed with 200 cc. of distilled water and steam distilled. Four 75-cc. fractions were collected. The first three fractions had rancid odors. Fraction 4 had an aromatic odor and contained green oily globules floating on the surface. Each fraction was shaken four times with 25-cc. portions of chloroform. The chloroform shakings from each fraction were combined and the solvent removed by distillation. This treatment gave an aqueous solution of material not removed by shaking with chloroform and a residue of chloroform-soluble material. The residue was mixed with water and titrated with standard sodium hydroxide solution. The neutralized liquid was shaken out with chloroform which removed material (volatile oil) which had not reacted with the sodium hydroxide. The neutralized solution was then evaporated to dryness and the residue, consisting of the sodium salts of the fatty acids, weighed.

The aqueous solution of material not removed by shaking with chloroform was likewise titrated with sodium hydroxide solution, evaporated to dryness and the residue weighed.

Qualitative tests performed on the sodium salt residues indicated the absence of formic and acetic acids.

The quantitative results obtained with the steam-volatile portion of Fraction A are tabulated below.

TABLE I.—ACIDS EXTRACTED FROM STEAM DISTILLATE BY CHLOROFORM.

	Cc. of *N*/1 Alkali Used.	Gm. of Sodium Salt Obtained.	Gm. of Sodium Salt Equivalent to Alkali Used.			
			Acetate.	Butyrate.	Caproate.	Caprylate.
Fraction I	10.889	1.3565	0.8930	1.1979	1.5039	1.8088
Fraction II	9.567	1.2326	0.7845	1.0524	1.3213	1.5892
Fraction III	5.640	0.7149	0.4625	0.6204	0.7789	0.9368
Fraction IV	3.500	0.4729	0.2870	0.3850	0.4834	0.5814

The results obtained with Fraction I point to a mixture of butyric and caproic acids in almost equimolecular proportions although a mixture of butyric, caproic and caprylic acids in certain proportions would probably correspond to the values given by Fraction I. Fractions II,

III and IV also point to a mixture of butyric and caproic acids with the amount of the higher acid steadily increasing.

Silver salts were prepared from the sodium residues and the per cent of silver in the salts determined. The results are tabulated below.

TABLE II.

	Per Cent Silver in Silver Salt.		Per Cent Silver in Silver Salts of.
Fraction I	50.3	Butyric acid	55.3
Fraction II	48.1	Caproic acid	48.4
Fraction III	46.9	Caprylic acid	43.0
Fraction IV	43.6	Capric acid	38.7

The conclusion as to the composition of Fraction I, Table II, is approximately the same as with Fraction I, Table I; however, since the silver percentage is lower than the percentage required of an equimolecular mixture of butyric and caproic acids the presence of a higher acid, probably caprylic, is indicated. Fractions II, III and IV show a continuously decreasing silver content indicating the presence of a larger ratio of the higher fatty acids.

The quantitative results obtained with the aqueous solution of material not removed by shaking with chloroform are tabulated below.

TABLE III.—ACIDS NOT EXTRACTED FROM STEAM DISTILLATE BY CHLOROFORM.

	Cc. of *N*/1 Alkali Used.	Gm. of Sodium Salt Obtained.	Gm. of Sodium Salt Equivalent to Alkali Used.		
			Acetate.	Butyrate.	Caproate.
Fraction I	1.284	0.1467	0.1053	0.1412	0.1773
Fraction II	1.260	0.1391	0.1033	0.1386	0.1740
Fraction III	1.007	0.1100	0.0826	0.1108	0.1391
Fraction IV	1.007	0.1154	0.0826	0.1108	0.1391

All four of the above fractions, which contain the volatile fatty acids not extracted from an aqueous solution by chloroform, point consistently to the presence of butyric acid.

Pure silver salts could not be prepared from the above sodium residues.

That portion of Fraction A which was not volatile with steam was separated from the water and treated by the lead-ether method for the separation of liquid from solid fatty acids. The method used was that of Tortelli and Ruggeri described by Rosenthaler (24). The ether-soluble and ether-insoluble lead salts were decomposed with 10 per cent hydrochloric acid and shaken out with ether. The ether solutions were washed repeatedly with water and were then dried with anhydrous sodium sulfate. The ether was distilled off from each fraction yielding a residue representing the solid acids and a residue representing the liquid acids.

Isolation of Myristic Acid.—Crystallization from various solvents of the material obtained from the ether-insoluble lead salts, *i. e.*, the solid acids, was attempted but without success. The solvents were removed and the residue amounting to approximately 20 Gm. was distilled under a pressure of 20 mm. Fraction I (up to 105° C.) consisted of a few cubic centimeters of a mixture of the various organic solvents which had been used in attempted crystallizations. Fraction II (up to 150° C.) consisted of half a cubic centimeter of a yellow liquid which could not be characterized. Beyond 150° C. the temperature rose very rapidly to 200° C. Fraction III, therefore, consisted of material distilling between 200° C. and 220° C. and amounted to 5 cc. Fraction IV distilled between 220° C. and 260° C. and amounted to about 3 cc. Decomposition, producing a red-brown distillate, began above 260° C. The residue in the distillation flask was a black-brown, viscous mass from which nothing could be isolated.

Fractions III and IV were both almost colorless liquids which solidified on cooling. Fraction III on crystallization from 95 per cent alcohol melted at 51–52° C. Fraction IV on crystallization from 95 per cent alcohol melted at 52–53° C. The two fractions were combined and recrystallized from alcohol until the melting point became constant at 53.5° C. The pure acid weighed 0.8 Gm.

$C_{14}H_{28}O_2$ has been reported as melting at 53.7° C. (25).

0.7722 Gm. of the acid required 33.11 cc. of 0.1*N* potassium hydroxide solution for neutralization. The molecular weight calculated from this data is 233.2.

$C_{14}H_{28}O_2$ = 228.2.
The silver salt was prepared and analyzed.
0.2730 Gm. of silver salt gave 0.0946 Gm. of silver.
Ag = 34.65 per cent.
0.1730 Gm. of silver salt gave 0.0601 Gm. silver.
Ag = 34.74 per cent.
Average: Ag = 34.70 per cent.
$C_{14}H_{27}O_2Ag$ requires Ag = 33.6 per cent.

The acid was obtained by distillation under a pressure of 20 mm. between 200° C. and 260° C.

$C_{14}H_{28}O_2$ is reported (26) as distilling at 196.5° C. at 15 mm.

The above-described compound was thus identified as myristic acid.

The material obtained from the ether-soluble lead salts, *i. e.*, the liquid acids, was also distilled under a pressure of 20 mm. Only a few drops distilled over up to 105° C. Between 105° C. and 250° C., 2–3 cc. of a slightly red-colored liquid were obtained. This fraction was discarded. Fraction III, distilling between 250° and 258° C., was a yellow liquid amounting to about 8 cc. No further distillate could be obtained without decomposition. The residue in the distillation flask was a black tarry mass from which nothing could be isolated.

Fraction III was dissolved in neutral 95 per cent alcohol and titrated with $N/1$ sodium hydroxide solution of which 14.805 cc. were consumed. The solution was evaporated to dryness and the residue dried to constant weight at 105° C. The residue weighed 3.5935 Gm. The molecular weight of the sodium salt calculated from the above data is 242.7 from which the molecular weight of the acid becomes 220.8.

The sodium salt was converted into the silver salt and the silver salt analyzed.

0.5862 Gm. gave 0.2060 Gm. Ag : Ag = 35.14 per cent.
0.3651 Gm. gave 0.1289 Gm. Ag : Ag = 35.31 per cent.
Average: Ag = 35.22 per cent.

The free acid was regenerated by treating a portion of the silver salt with 10 per cent nitric acid and shaking the mixture out with chloroform. The combined chloroform solutions were washed thoroughly with water and the chloroform evaporated off. The resulting yellow oil was dried over sulfuric acid and the iodine number determined.

0.2432 Gm. of the oil absorbed 0.05673 Gm. of iodine. The iodine number calculated from the above is 23.32. The iodine number of oleic acid, $C_{18}H_{34}O_2$, is 89.96. The low value obtained indicates that the oil was a mixture consisting largely of saturated acids.

The high silver content points to the presence of lower fatty acids, possibly capryldic, $C_8H_{16}O_2$ (per cent Ag in silver salt = 43.0) and capric, $C_{10}H_{20}O_2$ (per cent Ag in silver salt = 38.7). Silver oleate, $C_{18}H_{33}O_2Ag$ = 27.7 per cent Ag.

The low molecular weight, 220.8, likewise indicates the presence in the oil of lower fatty acids.

Oleic acid, $C_{18}H_{34}O_2$ = 282.3.

ALCOHOL EXTRACT.

The marc from the petroleum-benzin extraction was freed of solvent and exhausted in a Lloyd extractor with hot 95 per cent alcohol. Most of the alcohol was recovered in the Lloyd extractor. The concentrated extract was dried *in vacuo* over calcium chloride. The weight of the alcoholic extract thus obtained was 669 Gm. representing 7.43 per cent of the drug. The extract was a very thick green mass. It was incorporated intimately with the aid of alcohol with purified sand and sawdust. The sand and sawdust used had been previously extracted successively with petroleum benzin, ether, chloroform and alcohol. The mixed material was spread out in a thin layer and the alcohol allowed to evaporate at room temperature. The dry residue was packed in a percolator and extracted successively with benzin, ether, chloroform, ethyl acetate and 95 per cent alcohol. In each case percolation was continued until one liter of percolate gave no appreciable residue on removal of the solvent.

Petroleum Benzin-Soluble Portion of Alcohol Extract.—This extract was a dark green, soft mass weighing 64 Gm., representing 9.6 per cent of the alcohol extract. Two cubic centimeters of volatile oil were separated by steam distillation but no other definite substance could be obtained.

A portion of the extract was hydrolyzed by refluxing it for 2 hours with a 5 per cent solution of sulfuric acid in alcohol. Hydrolysis did not liberate a reducing sugar; therefore, material of a glycosidal nature was not present in the extract.

Ether-Soluble Portion of Alcohol Extract.—The residue after removal of the solvent was a dark green viscous liquid and weighed 42 Gm., representing 6.3 per cent of the alcohol extract. Thirty grams of the extract were dissolved in 95 per cent alcohol and allowed to stand. A green precipitate was thrown down. This was filtered off, dissolved in ethyl acetate and the solution again allowed to stand. About 2.5 Gm. of green material was deposited from the ethyl acetate solution. The green material was probably chlorophyll although it did not give the "brown phase" reaction (27). This reaction is given only by intact chlorophyll, that is, chlorophyll which has not undergone changes in its lactam groups.

The alcohol solution from which the chlorophyll had been deposited yielded on further treatment volatile oil and a soft resin. Here also there was no material of a glycosidal nature.

Chloroform-Soluble Portion of Alcohol Extract.—This extract was a green-black liquid weighing 31 Gm., representing 4.6 per cent of the alcohol extract. Only intractable resinous material was obtained from the extract.

Ethyl Acetate-Soluble Portion of Alcohol Extract.—This extract was a dark brown solid weighing 78 Gm., representing 11.7 per cent of the alcohol extract. The extract was washed with 95 per cent alcohol. There remained undissolved several grams of yellow-white crystals. These were found to be soluble in water and were recrystallized from water. The last crystallization yielded symmetrical cubes. The crystals were inorganic. Qualitative tests revealed the presence of sodium, potassium and chloride ions. Gravimetric and volumetric tests with silver nitrate indicated that the crystals were a mixture of sodium and potassium chlorides in the ratio of 80 parts of sodium chloride to 20 parts of potassium chloride.

The portion of the ethyl-acetate extract which dissolved in 95 per cent alcohol did not yield any crystalline material. The alcohol was removed, the residue mixed with water, and the mixture steam distilled. The distillate contained volatile oil and butyric acid.

The non-volatile material in the distilling flask consisted of a milky yellow supernatant liquid and a soft black resin.

Nothing could be isolated from the resin.

The supernatant liquid deposited a red-black solid on standing. This solid was soluble in 10 per cent sodium hydroxide solution and was reprecipitated from the alkaline solution on acidification. It may have been a phlobaphene.

After the deposition of the above substance the supernatant solution had a clear amber color. The solution gave a heavy black-green precipitate with ferric chloride solution, a cream-colored precipitate with strychnine sulfate, yellow precipitates with lead acetate and lead subacetate solutions, a white precipitate and a red ring at the interface with sulfuric acid, a brown precipitate with bromine water, a gray precipitate with gelatin solution and a white precipitate turning dark brown with potassium dichromate solution. The solution was strongly acid to litmus, had an astringent taste and assumed a dark brown color on alkalinization with ammonia. It reduced Fehling's solution. With ferric chloride solution, a very dilute solution of the substance produced a green precipitate which turned violet on addition of sodium acetate solution. The above reactions indicated the presence of a catechol tannin.

Concentration of the remaining tannin solution under reduced pressure resulted in the production of a brown uncrystallizable syrup. On being kept over sulfuric acid in a desiccator the syrup was converted into an amorphous brown powder. This was set aside for further investigation.

Portion of Alcohol Extract Not Dissolved by Petroleum Benzin, Ether, Chloroform and Ethyl Acetate.—The alcoholic extractive which remained in the sand and sawdust was removed with 95 per cent alcohol. Most of the alcohol was recovered from the percolate. The thick brown-black residue was mixed with water and the volatile oil removed by steam distillation.

The brown supernatant liquid in the distilling flask gave no definite reactions. The insoluble brown mass in the distilling flask was dissolved in alcohol and allowed to stand. About

4 Gm. of greasy, green material was deposited. This was filtered off and the alcoholic filtrate concentrated to the consistency of a thin syrup and poured slowly into a large volume of hot water. The precipitated resin was allowed to settle and the supernatant liquid decanted. The resin was redissolved in alcohol and reprecipitated. It was collected and dried. The dry resin amounted to 84 Gm. It was a black brittle solid with a smooth, shiny fracture. It had a slightly astringent taste and burned with a smoky flame. It was freely soluble in 10 per cent ammonia and in 6 per cent potassium hydroxide solution. Its acid value was 116.3; its saponification value, 227.4. It lost 6.6 per cent of its weight on being dried to constant weight at 105° C. It had an ash content of 0.32 per cent.

PHYSIOLOGICAL TESTS.

One cc. per Kg. of a fluidextract was given orally by capsule to two pregnant cats. No action was observed within 24 hours.

The marc remaining from the preparation of the fluidextract was exhausted by percolation with 50 per cent alcohol. This percolate was combined with the remainder of the fluidextract (the fluidextract had been prepared with 95 per cent alcohol as the menstruum). The combined extractives were concentrated as far as possible by means of a current of air at room temperature. The viscous resinous residue amounted to 16 Gm. Of this residue 1.8 Gm. was given orally by capsule to a female cat weighing 2.55 Kg. This represented a dose of 4.4 Gm. of drug per Kg. No effects were observed within 72 hours.

The remaining 14.2 Gm. of residue were mixed with alcohol and water to give a mixture which could be injected intraperitoneally. The total volume of the mixture was 40 cc. Five cubic centimeters were injected intraperitoneally into a female cat weighing 2.6 Kg. The dose of drug per Kg. calculated from the above was 4.3 Gm. No action was observed within 24 hours.

SUMMARY AND CONCLUSIONS.

1. A short botanical description of *Ipomea Pes-Caprœ* has been given, followed by reports of medicinal uses.

2. The U. S. P. X "constants" for crude drugs have been determined for *Ipomea Pes-Caprœ*.

3. Tests on small amounts of the drug for the presence of alkaloids were negative.

4. The total resin content of the drug was found to be 7.27 per cent.

5. The ash of the drug contained the sulfate, chloride and carbonate radicals and the following metals: tin, iron, aluminum, magnesium, calcium, sodium and potassium. Silica was also present.

6. The drug yielded 0.048 per cent volatile oil. The oil distilled between 127° and 145° C. at 20 mm.; specific gravity 28° C./28° C. = 0.9626; refractive index at 26° C. = 1.4703.

7. Extraction of 9 Kg. of the drug with petroleum benzin yielded 3.91 per cent of extractive. The following substances were isolated from the extract: pentatriacontane; triacontane; a sterol: $C_{29}H_{49}OH$; behenic acid, melissic acid, butyric acid and myristic acid. The presence of glycerol, of saturated fatty acids of low molecular weight and of an unsaturated fatty acid was demonstrated.

8. The marc from the petroleum-benzin extraction was percolated with 95 per cent alcohol and yielded 7.43 per cent of extractive. The extract yielded volatile oil; butyric acid; chlorophyll; resin; inorganic crystals consisting of sodium and potassium chlorides; and a catechol tannin.

9. During the examination of the drug various extractives were administered to cats but in no case was there an apparent response to the drug.

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PREPARATION OF RESIN OF PODOPHYLLUM.*

BY A. H. UHL.[1]

According to Lloyd (1), King was the first to have prepared the so-called resin of Podophyllum. The discovery was accidental. Its precipitation resulted when the hydroalcoholic extract was mixed with an infusion of the same drug (2). The earlier work on the "resin" threw no light on its chemical composition except in so far as the observation of its solubility in hot water caused Maisch to express the opinion that it was not a true resin (3). As the therapeutically important constituent of the drug, its preparation and its properties received special attention, more particularly when, in 1860, the resin of Podophyllum was made official in the United States Pharmacopœia.

EXPERIMENTAL.

The powdered dried roots and rhizomes of *Podophyllum peltatum* were extracted to exhaustion with 95 per cent alcohol in a Lloyd extractor. A portion of the alcohol was recovered leaving a syrupy, nearly black extract. On standing over night a greenish oily-looking material

* Presented before the Scientific Section, New York meeting, 1937.

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separated. To remove this material and obtain any additional material which might not have separated, the extract was washed several times with petroleum ether. This removed the oily substance.

The concentrated alcoholic extract was slowly poured into a 1 per cent hydrochloric acid solution which had been cooled to 10°. The precipitate thus obtained was washed three times by decantation, placed on a force filter and sucked dry, finally washed well with cold water. The product was allowed to dry at room temperature. It was noted that the dried product prepared in this manner did not agglutinate when allowed to stand in a warm room. Inasmuch as fatty material as well as the resin is precipitated when the concentrated alcoholic extract is poured into acidulated water, it seems desirable to remove the fat previous to the precipitation of the resin. The resin thus prepared answered all the requirements of the U. S. P. XI, and of the several batches prepared at various times, not once did coalescence or agglutination appear.

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(2) J. U. Lloyd (*Am. J. Pharm.*, 62, pages 242, 386, 605) has pointed out that the substance obtained by Hodgson (*Am. J. Pharm.*, 3, page 273) from the dried root by the use of hot water, cannot be the same substance that is now obtained by the extraction with alcohol. He gives King the credit for being the first to obtain and recognize the true resin in 1835. (See King's letter to Lloyd, above reference.) Power (PROC. A. PH. A., 25, 420 (1877)) decided from his own researches that the substance obtained by Hodgson could have been none other than the resin as we now know it. He considers the inactivity of Hodgson's product to be due to the observed effect of alkalies upon the resin in diminishing the activity. Abenthy (*Am. J. Pharm.*, 33, 299 (1861)) followed in detail the method of Hodgson, and showed that the product contained a certain amount of the active property of the drug.

(3) *Am. J. Pharm.*, 46, 231 (1874).

PHARMACODYNAMICS OF THE CARDIOACTIVE PRINCIPLES OF URGINEA MARITIMA (SQUILL).*

BY DAVID ROBERT CLIMENKO, M.D., PH.D.

Squill (Urginea maritima) has played a peculiar rôle in the therapeutic armamentarium. The ancients regarded it as one of their most thoroughly understood drugs and accorded it a high place among their therapeutic agents. It was familiar to the Egyptian, the Arabian and the Greek schools of medicine, and descriptions of it are to be found in the Ebers papyrus, in the works of Epimenedes, Dioscorides, Hippocrates and Galen. The drug did not survive the classical period and fell into complete desuetude during the middle ages. But it is not the purpose of this study to discuss the history of the use of this drug, accounts of which may be found in the works of Joz (1), Chamberlin and Levy (2) and Scheer and Sigerist (3).

It is not difficult to account for the almost complete abandonment of this once highly respected drug. The crude substance contains an extremely variable concentration of therapeutically active principle together with a mass of highly irritating resinous substances. The presence of these irritants makes it almost impossible to administer a therapeutically effective dose without inducing nausea, diarrhoea and irritation of the upper respiratory tract. The isolation of the cardioactive principles from squill started with the pioneer work of Vogel (4) who, in 1812, described "scillitine" and Thomson (5) who described "scillitite" in 1831. It was

* From the Pharmacological Laboratory, Calco Chemical Company, Inc., Bound Brook, N. J.

not until Stoll (6) isolated scillaren and Dyas (7) described a method for the extraction of crystalline "Urginin A" and amorphous "Urginin B"[1] from the crude drug that a practical method was made available for the isolation and preparation of the cardioactive principles of squill.

It is with the pharmacodynamic action of a mixture of Urginin A and Urginin B that this study is primarily concerned, and the work reported here has been carried out on a preparation known as Urginin.[2]

Urginin was obtained in the form of compressed lactose tablets containing 0.5 mg. of the active principle in each tablet. For intravenous administration, for assay purposes or for perfusion purposes, the active principle was prepared in the following manner. Three hundred tablets were weighed in batches of twenty in order to establish the average tablet weight. The tablets were then ground in a mortar until all of the powdered material passed through a 60-mesh sieve. Powder equivalent in weight to 40 tablets (20 mg. Urginin) was placed in a glass-stoppered Erlenmeyer flask to which 40 cc. of alcohol and 20 cc. of glycerine were added. After agitation in a mechanical shaker for about twelve hours the contents of the flask was transferred to a 1000-cc. volumetric flask and made up to volume with normal saline solution. This suspension was well shaken and centrifuged. The supernatant liquid was decanted and the lactose residue discarded.[3] This slightly opalescent supernatant fluid which contained 0.002% of active principle formed the basis for all solutions used in experimental work or in assaying the drug. Fresh solutions were always employed and watery solutions were always discarded after twelve hours.

The other drugs used in this study were official samples of ouabain obtained from the Bureau of Food and Drugs of the U. S. Department of Agriculture, a preparation of the active principles of Digitalis lanta (digoxin, Burroughs Wellcome), a sample of digitoxin (Merck) and a Tincture of Digitalis (Lilly). All solutions for experimental administration were made up to contain the same percentage of alcohol and glycerine as the final Urginin solution.

All assays were carried out on cats, the rate of injection being adjusted so that death occurred from 55 to 65 minutes after the initiation of the intravenous administration. Ten animals were used in each assay.

Uniformity and Stability.—One of the most desirable features of a cardiac glucoside for therapeutic use is uniformity of potency. In actual practice, all samples of cardiac glucosides designed for therapeutic purposes are subjected to

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[1] Originally known as "Scillonin A" and "Scillonin B."

[2] Urginin is described in N. N. R. as a mixture of two water-insoluble glucosides, derived from squill, in the proportions in which they exist in the drug; namely, about equal parts. The drug was supplied by the Calco Chemical Co., Inc., Bound Brook, N. J.

[3] The completeness of this extraction process was once questioned. To clear up this point, the residue from 200 Gm. of tablet material was obtained after following the procedure outlined above. This residue was transferred to a Soxhlet and extracted with ethyl acetate for twelve hours. The ethyl acetate was removed by evaporation in a current of warm air and the extracted material taken up in 5 cc. of absolute alcohol and glycerine. This alcoholic solution was made up to 200 cc. with normal saline and examined for the presence of pharmacologically active substances on the isolated turtle heart, with a resultant demonstration of the presence of traces of a cardioactive principle. The amount, however, was so small that it was not amenable to mensuration by the cat method.

bio-assay and those which fail to fall within certain arbitrary limits are either discarded or adjusted so that they will meet these requirements. During the course of the last two years, thirty-nine samples of Urginin have been assayed by the Hatcher cat method. The results of these assays are compared with a series of twenty-eight assays carried out by the same method on official samples of ouabain.

TABLE I.

Drug.	Number of Assays.	C. U. per Mg.	Lower Limit.	Upper Limit.	Standard Deviation.	Probable Error of the Mean.
Urginin	39	4.16	3.47	4.83	±0.2	±0.026
Ouabain	28	9.48	8.47	11.11	0.32	0.035

This uniformity of potency is even more striking when it is realized that the above comparison is made between an extended series of manufacturer's batches, and a number of official ouabain samples with assays carried out during every month of the year. It should be pointed out that the assay value for ouabain falls slightly below the value usually given.

A second requirement, almost as important as uniformity, is stability. None of the cardiac glucosides are stable substances, but the degree of instability varies greatly, particularly with regard to the conditions under which the glucosides are kept. The cardioactive principles of urginin are very susceptible to hydrolytic change, and aqueous suspensions are very unstable. The lactose tablets, or anhydrous alcoholic solutions, however, are stable. The following table shows the results of a series of assays made on three samples of the drug at intervals of six months. The tablets were kept in ordinary glass containers fitted with screw tops. No attempt was made to exclude air.

TABLE II.

Sample No.	Date Assayed.	C. U. per Mg.	Standard Deviation.	Probable Error of the Mean.
1117	7/2/36	4.22	±0.092	±0.011
(Tablets)	12/8/36	4.14	0.032	0.007
	6/5/37	4.02	0.026	0.007
	12/2/37	4.12	0.045	0.010
1101	2/20/36	4.01	0.024	0.005
(Solution)	9/5/36	3.85	0.029	0.006
	3/11/37	3.80	0.065	0.014
1111	2/5/36	4.38	0.026	0.0071
(Tablets)	8/14/36	4.10	0.053	0.012
	3/12/37	4.18	0.065	0.015
	7/14/38	4.14	0.024	0.0053

Pharmacodynamic Action.—It has been pointed out by Cushing (8), by Clark (9), by Jenny (10) and by Mendel (11) that the general pharmacological action of the cardioactive principles of squill is fundamentally the same as that of digitalis. The end result of the slow intravenous administration of a fatal dose of the two substances to test animals is so similar that it is almost impossible to distinguish them from one another. These effects have been described in detail by Joz and Wallace and Van Dyke (13). The therapeutic effects in cardiac disease are similar, as has been shown by Markwalder (14), Massini (15), Carr and Mayer (16), Chamberlin and Levy, and Chauncey and Maher (12).

There are, however, a number of differences between the action of Digitalis and Urginin that are not brought out by a study of the effects of the slow intravenous administration of a fatal dose of the drug. The principal difference, both from a pharmacodynamic and therapeutic point of view is to be found in the cumulative effect of the two drugs.

Cumulation: The rate of excretion or inactivation of the cardioactive principles of squill is much more rapid than that of digitalis or strophanthus. Wallace and Van Dyke (13) have shown that the drug is only half as potent a cumulative poison as ouabain and less than one-third as potent as digitoxin when tested on dogs. Kwanichira-Okushima (17) has described similar results for the cat. Kingisepp (18), on the other hand, has described scillaren as having a much greater cumulative effect than digitoxin or adonis glucosides when tested on the frog.

This point has been investigated experimentally, using the principles laid down by Hatcher (19). Three samples of urginin, three samples of ouabain and four samples of digitoxin were assayed by the cat method. After establishing the potency of each preparation in terms of cat units, each of a large series of cats received an intravenous dose equivalent to 50% of the M. L. D. of the substance being tested. At intervals of 24, 48 and 72 hours after this initial administration, groups of animals were used for the assay of the drug. If all of the drug originally injected was still effectively present in the animal at the time of the assay, then it would be expected that 50% of the M. L. D. would be required to produce death. Therefore, the difference between the quantity of the drug required to produce systolic arrest of the heart and the expected 50% may be regarded as an index of the quantity of drug excreted or inactivated during the interval between the original administration and the subsequent assay. Thus, 72 hours after the administration of 50% of the M. L. D. of urginin, the effect of only 3.6% remains; after the same interval of time, the effect of 14% of ouabain and 28% of digitoxin remain. These facts are brought out in the following table.

TABLE III.

Drug.	Assay Value (C. U. per Mg.)	Per Cent Lethal Dose Administered.	No. of Animals.	Per Cent Lethal Dose to Produce Systolic Arrest after 24 Hours.	No. of Animals.	Per Cent Lethal Dose to Produce Systolic Arrest after 48 Hours.	No. of Animals.	Per Cent Lethal Dose to Produce Systolic Arrest after 72 Hours.	No. of Animals.
Urginin									
A	0.244	50%	15	69%	5	90%	5	96%	5
B	0.229	50%	15	83%	5	92%	5	97%	5
C	0.210	50%	15	77%	5	89%	5	94%	5
Ouabain									
D	0.109	50%	10	67%	4	78%	3	90%	3
E	0.106	50%	08	62%	2	80%	3	84%	3
F	0.110	50%	08	70%	3	84%	2	84%	3
Digitoxin									
G	0.301	50%	10	52%	4	55%	3	70%	3
H	0.314	50%	19	51%	3	57%	3	74%	3
I	0.308	50%	10	54%	3	54%	4	72%	3
J	0.310	50%	10	56%	3	59%	4	71%	3

Thus, at 72 hours, one might describe urginin as having one-fourth the cumulative effect of ouabain and one-seventh the cumulative effect of digitoxin. Unfortunately, different values are found for different time intervals. It would be very desirable to express this ratio in a quantitative manner. If the logarithm of the concentration of the drug remaining in the animal is plotted against time (see Fig. 1) it will be seen that an exponential relationship exists.

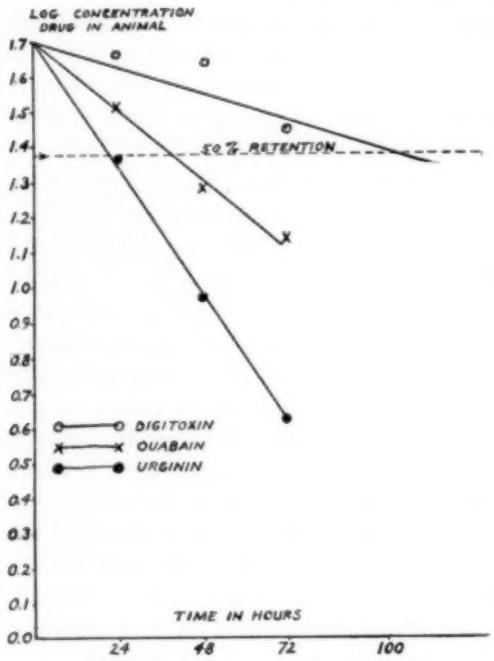

Fig. 1.

This relationship may be expressed in the following manner:

$$\log c = c_0 \left(\frac{1 - T}{B}\right)$$

Where c = concentration remaining in the animal
c_0 = log. of initial concentration
T = time
B = cumulative index.

In the three cases illustrated, the cumulative indices are:

Urginin	111
Ouabain	204
Digitoxin	595

A simpler method for quantitatively expressing the relative cumulative effects of these three drugs is to describe it in terms of time required for 50% of the amount of drug initially administered to disappear. Using this method, the following values are obtained for the relationship of the cumulative action of these three drugs:

Urginin	20
Ouabain	40
Digitoxin	100

Reversibility of Action.—Closely allied with the phenomenon of cumulation is the question of drug fixation and reversibility of action. Cumulation depends primarily on the rate of excretion or the rate of inactivation of a drug. This in turn is dependent on the degree of fixation which takes place between the drug and the physiological structure with which it is reacting. In the case of the cardioactive glucosides of strophanthus and digitalis, the myocardial fixation is very potent, much more so than is the case with the active principles of squill. This point has been demonstrated by Rothlin (20) and Graf (21), but Kingisepp (22) states that the reversibility of the squill reaction is about of the same order as ouabain.

If ouabain or digitoxin is applied to an isolated turtle heart until systolic arrest occurs it is practically impossible to reëstablish rhythmic contractions by washing with Ringer-Locke solution. On the other hand, systolic arrest resulting

from the application of the cardioactive principles of squill is readily reversible; the spontaneous rhythm may be reëstablished by washing with Ringer-Locke. Under these circumstances, subsequent applications of Urginin will produce systolic arrest in successively shorter periods of time and diminishing quantities of the drug will be required, while increasing periods of time will be required for Ringer-Locke washing to reëstablish spontaneous rhythm. These facts are brought out in the following table which shows the comparative effects of urginin, digoxin, digitoxin and ouabain on the isolated turtle heart.

TABLE IV.

Drug and Concentration.		Time Required to Produce Initial Systolic Arrest.	Time to Reëstablish Rhythm.	Time Required to Produce Second Systolic Arrest.	Time to Reëstablish Rhythm.	Time Required to Produce Third Systolic Arrest.	Time to Reëstablish Rhythm.
Urginin							
(1:100,000)	a	21.00 min.	10.50 min.	12.00 min.	18.50 min.	7.50 min.	42.0 min.
	b	20.25	12.25	11.50	24.00	1.50	...
	c	22.50	10.30	14.25	15.50	6.75	46.0
	d	24.50	11.50	9.00	18.75	5.00	48.0
	e	21.00	10.50	7.50	35.00	0.50	...
	f	19.25	12.50	11.00	24.00	1.75	54.0
	g	20.00	11.00	10.25	18.50	3.00	42.0
	h	22.50	10.50	8.50	15.25	6.50	38.0
Digoxin							
(1:50,000)	a	90.00	90.00	12.00	...	...	...
	b	94.00	...	...	...	...	...
	c	82.00	75.00	10.00	...	...	...
	d	105.00	...	...	...	...	...
	e	98.00	68.00	16.00	...	...	...
Ouabain							
(1:60,000)	a	32.00	20.00	9.00	65.00	4.50	...
	b	35.00	...	...	...	...	...
	c	35.00	18.00	7.00	90.00	2.00	...
	d	28.00	24.00	5.00	...	...	...
	e	26.00	21.00	11.00	...	...	...
Digitoxin							
(1:60,000)	a	32.00	68.00	12.00	...	...	...
	b	38.00	...	...	...	...	...
	c	42.00	...	...	...	...	...
	d	31.00	...	...	...	...	...
	e	30.00	...	...	...	...	...

It will be noted that the concentration of urginin in the above table is approximately one-half that of the other glucosides employed. This has been done in order to produce systolic arrest in approximately the same time interval. A 1:100,000 solution of Urginin produces systolic arrest in about twenty minutes, while a 1:60,000 solution of ouabain or digitoxin required about thirty minutes to produce the same effect. 1:50,000 Digoxin requires ninety minutes, but more concentrated solutions produce an irreversible change.

Emetic Action.—The emetic action of the cardioactive glucosides of digitalis and strophanthus has been the subject of a comprehensive investigation by Hatcher

and his co-workers (23), who have shown that this action is primarily the result of reflex stimuli arising from the neighborhood of the heart. It is common knowledge that during the course of the assay of the digitalis bodies by the intravenous cat method hypersalivation and vomiting occur.

It is, however, not equally well known that it is difficult to induce the vomiting mechanism with an animal in the supine position, the common position of an experimental animal used for slow intravenous injection. It has been our experience that vomiting occurs in about 75% of cases where such supine animals are receiving ouabain, and in 60% of the cases where they are receiving digitalis. In a series of 460 animals that have received urginin, our records show that vomiting occurred in six animals, or less than 2%. This discrepancy between the production of emesis by the cardioactive principles of squill and those of digitalis and strophanthus bear out the clinical experiences of the large number of investigators who have shown that therapeutic doses of the cardioactive principles of squill may be administered without the production of nausea or emesis in patients who always become nauseated or vomit after the administration of digitalis bodies.

The intravenous administration of 50% of the M. L. D. of urginin to a series of twenty cats in an unrestrained position produced emesis in only 40% of animals. The remaining 60% showed marked hypersalivation. Electrocardiographic records taken immediately before and 30 minutes after administration showed a marked bradycardia, with an increase in AV conduction time in all animals, and occasional ventricular extrasystoles in 10% of the animals.

The administration of 50% of the M. L. D. of ouabain to a series of twenty animals was followed by nausea and vomiting in every case, while electrocardiographic records taken thirty minutes after administration showed marked bradycardia, increased AV conduction time, and occasional ventricular extra-systoles.

The intravenous administration of 50% of the lethal dose of Tincture of Digitalis produced vomiting in 100% of cases, showing the same type of electrocardiographic changes as described above. In this series the bradycardia was not so marked as it was in the two preceding series.

After the administration of ouabain or digitalis vomiting occurred within three minutes of the completion of the intravenous administration. In those cases where emesis occurred after the administration of Urginin there was a delay of ten to eighteen minutes before the onset.

CONCLUSIONS.

1. Urginin is a mixture of two water-insoluble cardioactive glucosides of Squill, possessing a uniform biological potency and demonstrating a high degree of stability.

2. A method is described for the quantitative expression of the relative cumulative effects of cardioactive glucosides.

3. The cumulative effect of Urginin is half as great as that of ouabain and one-fifth as great as that of digitoxin.

4. The reversibility of the action of a series of cardioactive glucosides on the isolated turtle heart may be expressed in the following way: Urginin is more readily reversible than ouabain, which is more readily reversible than digoxin, which is more readily reversible than digitoxin.

5. The emetic action of Urginin on the intact cat is less than that of ouabain or Tincture of Digitalis.

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NOTE ON UROTROPIN MANDELATE.*

PREPARATION AND TOXICITY OF A NEW URINARY ANTISEPTIC.

H. G. KOLLOFF AND J. W. NELSON.

The recent discovery that mandelic acid (1) and certain of its salts (2), (3), (4) are efficacious in the treatment of urinary infections makes it desirable to study new salts and combinations of this acid. In this preliminary paper, we wish to report the preparation and toxicity of the hexamethylenetetramine salt which appears to offer possibilities as a superior mandelic acid preparation.

* From the Laboratory of the Upjohn Co., Kalamazoo, Mich.

EXPERIMENTAL.

A solution of 86 Gm. of mandelic acid and 79.2 Gm. of hexamethylenetetramine in 100 cc. of water was concentrated to a volume of 85 cc. and allowed to crystallize in the cold. The crystalline material (63 Gm.) was filtered and recrystallized from 200 cc. of absolute alcohol from which the product was obtained as clumps of small, white, granular crystals (54 Gm.) m. p. 130–132° C. The original mother liquor on further concentration and cooling yielded 88 Gm. more of the salt melting at 129–131° C.

Analyzed for $C_{14}H_{20}O_4N_4$

	C.	H.
Calculated for	57.53	6.85
Found	57.44	6.88

The urotropin mandelate is soluble in alcohol, very soluble in water (5 Gm. dissolve easily at room temperature in 5 cc. of water) and only slightly soluble in ether and acetone. The salt has a sour, not unpleasant taste, and the aqueous solution is slightly acid to litmus.

Samples of this material which have stood for a year show no signs of decomposition. Samples which have been exposed to light and air do, however, possess a slight odor of benzaldehyde.

TOXICITY.

Urotropin mandelate was administered orally by stomach tube in aqueous solution once daily for 20 days to male rats weighing approximately 100 Gm. at the start of the experiment. The rats were weighed daily and the dose administered was calculated per Kg. of body weight. A second group of rats served as controls and received orally by stomach tube 10 cc. of water per day per Kg. of rat. The results are summarized in Table I.

TABLE I.

Rat Number.	Material Administered.	Dose per Kg.	Initial Body Weight.	Body Weight after 20 Days' Dosage.	Gain in Body Weight.	Total Amount of Material Administered Per Rat.
		Gm.	Gm.	Gm.	Gm.	Gm.
21	Urotropic Mandelate	5	95.0	61.0	−34.0	7.18
22	in aqueous	5	106.0	*	....	....
23	solution	5	100.0	98.0	− 2.0	7.73
24	0.5 Gm. per cc.	5	100.0	84.0	−16.0	8.66
	Average of group	5	100.2	81.0	−17.3	7.86
1	Urotropin Mandelate	2	100.0	130.0	+30.0	4.66
2	in aqueous	2	96.0	124.0	+28.0	4.24
3	solution	2	97.0	103.0	+ 6.0	3.96
4	0.2 Gm. per cc.	2	99.0	138.0	+39.0	4.42
5		2	99.0	136.0	+37.0	4.58
	Average of group	2	98.2	126.2	+28.0	4.37
		Cc.				Cc.
6	Water controls	10	99.0	145.0	+46.0	24.3
7	Water controls	10	105.0	170.0	+65.0	26.9
8	Water controls	10	97.0	168.0	+71.0	26.6
9	Water controls	10	97.0	179.0	+82.0	27.8
10	Water controls	10	94.0	135.0	+41.0	24.6
	Average of group	10	98.4	159.4	+61.0	26.0

* Rat Number 22 died on 16th day of the experiment.

AUTOPSY.

No macroscopic abnormalities were noted in the group of rats receiving 2 Gm. urotropin mandelate per Kg. daily. However, ulcers of the pylorus, possibly due to the high concentration

of the solution administered, were found in two of the three rats receiving 5 Gm. of the compound per Kg. body weight daily. The pylorus was definitely thickened in all rats receiving the 5 Gm. per kilo dose.

More detailed experiments are in progress and will be reported at a later date.

SUMMARY.

1. The preparation of the hexamethylenetetramine salt of mandelic acid has been described.

2. The toxicity of the hexamethylenetetramine salt of mandelic acid has been determined in rats.

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PRICE PROBLEM WITH PHYSICIANS—U. S. P. AND N. F. SUGGESTIONS.*

BY EMMET WEAVER.[1]

I have often wondered whether or not pharmacists who operate stores other than those located in Medical Arts Buildings have the same problems that we do. My store is located in a building where more than three hundred doctors of all kinds have their offices. During the course of the day every one of these doctors passes into my store. Some are easy to contact, while others are very difficult of approach. Some are easily satisfied, while others seem never to be satisfied no matter how hard we try to please them.

Our arrangement is somewhat the same as in most stores, although in some instances we have departed from the orthodox and installed some innovations. The prescription department is located in a balcony, is connected with the main store by means of speaking tubes, and recently we have installed a loud speaker system which may be easily heard in all parts of the store. Three men are on duty in this department at all times and they have nothing before them but prescription problems.

We have on hand a complete stock of pharmaceuticals representing every ethical manufacturer and it is seldom we are without a preparation we should have. The biological department is complete in every respect and is equipped with probably one of the few mechanical refrigeration units in the southwest. This insures that the biologicals will have proper care under very trying conditions. I mention these things in order that you may have a picture of our most important department.

We have the problem of price to the physician. The average doctor will call for a certain preparation ostensibly for his own use. We quote a price of twenty-five per cent under list. Immediately we have the retort it is too high and can be obtained from so and so at such and such a price, which in most cases is way below

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* Presented before Section on Practical Pharmacy and Dispensing, A. PH. A., Dallas meeting, 1936.

[1] President, Texas Board of Pharmacy, Fort Worth.

what we can afford to make. Whether or not this is a ruse on the part of the physician concerned to beat our prices down, or can be attributed to the keen competition of other druggists who are striving for this business, is a question. This problem has caused us considerable concern, and we are at sea to meet this demand for a lower price than the one we quote.

Another problem is to have the physician give his patient a prescription and tell him "now this is a very simple remedy and should not cost you more than a certain small sum." As a matter of fact the preparation called for often is a proprietary which cannot possibly be dispensed at the price quoted by the physician. Do we as pharmacists, when we send a patient to the physician, tell that patient, "he will only charge you a dollar," or something of like nature? Would the physician not immediately call the store and ask upon what authority we told the patient his charges would be so much? It seems just as fair for one as the other and no physician should quote a price, especially when he knows nothing whatsoever about the actual cost of the article mentioned.

Then again the physician gives the patient a sample left by some detail man, with the inference that the preparation costs little or nothing and is glad to furnish it as part of his treatment. A little later the patient comes into the store with the empty bottle, and asks for a refill. When presented with the prescription, the patient is astounded to learn that it costs so much. He refuses to take it, saying that he will wait and see the doctor the next day and get another free bottle. In a great many cases, this hurts the ethical business of the pharmacist and lowers him in the estimation of the public. The problem is how best to meet a situation of this sort.

While on the subject of detailing, I have found that if we keep one man constantly calling on the physicians in the building, detailing N. F. and U. S. P. preparations, we gradually bring the fair-minded members of the medical profession back to ethical pharmacy. The many preparations recognized by these authorities enable us, in many instances, to convince the physician that it is possible to dispense at a saving to the patient and at the same time giving the physician a preparation whose action he may absolutely depend upon. How many pharmacists have tried this and would it not be a good idea for the AMERICAN PHARMACEUTICAL ASSOCIATION to inaugurate a campaign of this sort over the entire United States? The average physician is too prone to listen to the detail men, with the result that the art of prescription writing is fast disappearing. This is particularly true of the younger doctors—they have little or no knowledge of how to write a prescription and naturally take the easiest course. It might be well for this body to bring the matter up for consideration by the American Medical Association in connection with a course of prescription writing to be added to the curricula of the medical schools.

Then there is the dispensing physician. Of course we are in a measure powerless to control this problem. I feel, however, that by means of a campaign of instruction, and instilling a greater degree of confidence among the members of the Medical profession, it will be possible for us to coöperate in such a way that the danger of this practice will be minimized.

There are many other problems in the operation of a Medical Arts store. I trust the questions I have raised will bear some thought, and that effort will be made, generally, to control the problems.

A STUDY OF EMULSIONS.*

BY P. T. REES.[1]

The word Emulsion is derived from the Latin "Emulsus," meaning "To milk out." This class of medicaments is one of the oldest of pharmaceutical preparations, many products of this nature being in use before their structure was understood. Patent number 327,229 was issued to John Canrick in about 1884, covering a method for their manufacture.

In the light of modern research the complexity of the structure of emulsions has led to various definitions, but for pharmaceutical purposes they may be satisfactorily described as "Homogeneous products of two or more immiscible substances held by the intervention of an intermediate agent."

Again, from a pharmaceutical standpoint, emulsions of two types only need be considered, namely, Oil-in-Water and Water-in-Oil, with practically all of those intended for internal use being the Oil-in-Water type. All emulsions consist of three (3) parts or phases: (1) The continuous phase. (2) The dispersed phase. (3) The intermediate phase. In the Oil-in-Water type, the water being the continuous phase, oil the dispersed phase and the emulsifying agent the intermediate phase.

Many theories have been suggested for the behavior of these phases in the formation of emulsions:

(1) The Viscosity Theory which attempts to explain the stability of these products by a hindrance to coalescence due to the increased viscosity of the mixture.

(2) The Surface Tension Theory which states that emulsification is made possible by equalization of the surface tensions of the two liquid phases by intervention of the intermediate phase. In this manner inhibiting coalescence.

(3) The Hydration Theory which proposes that emulsification may occur only when the continuous phase can form hydrated compounds with the intermediate agent.

(4) The Adsorbed Film Theory which is, at present, the most generally accepted explanation and places the emulsifying agent as the third or intermediate phase. It advances the concept of adsorption of this phase, as a film about the oil, with both the continuous and the dispersed phases wetting this adsorbed film forming a difference in surface tensions on its two sides, thus causing it to bend to the side with the higher surface tension, becoming concave, and tending to enclose the liquid on that side. The mono-valent soaps, acacia, etc., which are more readily dispersed in water than in oil will enclose the oil globules and form the Oil-in-Water type emulsion. Di- and tri-valent soaps being more readily dispersed in oil will enclose the water and form the Water-in-Oil type emulsion.

The use of the Oil-in-Water type emulsion for internal administration is obvious, since water is the continuous phase, the finished product will be miscible with the aqueous body fluids, thus overcoming the objectionable taste of the oil phase.

In the field of Dermatology both types are employed but more commonly the Water-in-Oil. In the cosmetic industry approximately one-third of all items are in the form of emulsions. From the standpoint of these two fields alone, emulsions present not only efficacious preparations but also materially improve the appearance, usefulness and stability of these products.

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* Presented before the Section on Practical Pharmacy and Dispensing, A. PH. A., New York meeting, 1937.

[1] U. S. Naval Medical Supply Depot, Brooklyn, N. Y.

It is realized that the practical pharmacist rarely compounds extemporaneous prescriptions for emulsions, nevertheless he deals in many such products and is interested in their preparation and stability. The article by O. U. Sisson in the *Journal of the National Association of Retail Druggists* of June 1936, describes the modern technique for preparing emulsions and recommends the emulsification of many prescriptions which are frequently dispensed in a condition unsatisfactory to the physician, pharmacist and patient. His suggestions cover not only the eight official and various non-official emulsions but many ointments, dermatological prescriptions and professional cosmetics.

In general there are four (4) methods employed in Pharmacy for preparing emulsions, *e. g.*, trituration in a mortar, agitating in a milk-shake mixer, stirring in a mechanical mixer and recently by use of a Homogenizer. In investigating the practicability of the Homogenizer for pharmaceutical purposes it was decided to study the efficiency and speed of the other methods of manufacturing emulsions, out of which grew the material for this paper.

A hand-operated Homogenizer of about twelve (12) ounce capacity, suitable for small scale work, has recently been offered to the trade by International Emulsifiers, Inc., at a cost of $4.95. The apparatus is well constructed, durable and efficient. Tice, in the *American Journal of Pharmacy* of April 1935, reported on the advantages of the Homogenizer and compared products made with it to those prepared by the usual trituration method.

The present study has attempted to compare all of the generally used methods of emulsification on the basis of time, efficiency, economy, palatability and stability. The exhibit clearly shows the difference in stability of the products obtained by the four methods and their stability as related to the degree of dispersion of the dispersed phase as demonstrated by the micro-photographs.

Emulsion of Cod Liver Oil, U. S. P. Eleventh Revision, was chosen for these experiments since it is widely used, due to the present public demand for vitamin therapy, and the possibility of demonstrating the palatability of the finished products was considered.

Two hundred fifty (250) cubic centimeters of emulsion were prepared by each of the four methods of manufacture. In all cases the acacia was first mixed with the oil in starting the preparations and the alcohol added as a preservative to the finished product.

SPECIMEN "A" (HOMOGENIZER).

A primary emulsion was first prepared by passing the acacia in oil and water through the machine three (3) times. The syrup, methyl salicylate, final water and alcohol then added to the primary emulsion and the total volume again passed through the machine three (3) times.

Total time of manufacture being eight (8) minutes.

A creamy white preparation was obtained which showed no signs of stratification until after one (1) month. After ten (10) months of ordinary shelf-storage stratification may be noted at the bottom of the container. Approximately twenty (20) cc.

The product is noticeably free from included air-bubbles and shows a very uniform, finely divided dispersion of the oil phase.

SPECIMEN "B" (MORTAR).

The Continental Method, as specified in the U. S. P. Eleventh Revision, was used in the manufacture of this product.

Total time of manufacture being sixteen (16) minutes.

The fresh preparation appeared very satisfactory, being slightly more yellow and fluffy than Specimen "A" due to air enclosure during the trituration. After one (1) week there was evidence of stratification with about thirty (30) cc. of separation at the bottom of the container. After one (1) month stratification was decidedly apparent with the primary emulsion occupying about two-thirds of the container. Little change was noted between the one- (1) and ten- (10) month storage period.

The product shows oil globules of irregular sizes and of a fairly coarse and uneven dispersion.

SPECIMEN "C" (MILK-SHAKE MIXER).

The primary emulsion was prepared in the shaker and the balance of the ingredients added.

Total time of manufacture being sixteen (16) minutes.

A three- (3) layer stratification was noted within twelve (12) hours.

It is believed that but very little actual emulsion was formed by this method of manufacture, it being the thin white central layer of the finished product which shows an uneven dispersion of the oil globules and difficulty is experienced in identifying the continuous or the dispersed phase.

SPECIMEN "D" (MIX-MASTER).

The primary emulsion was first prepared in the mixer and the other ingredients added.

Total time of manufacture being eight (8) minutes.

The freshly prepared product appeared very satisfactory; the color and viscosity approaching that of Specimen "B." Stratification of approximately four (4) cc. appeared after one (1) week storage. After one (1) and ten (10) months' storage the stratification approximated that of Specimen "B."

The product shows a more uniform oil dispersion than seen in Specimen "B" but the size of the particles is larger than those of Specimen "A."

An opportunity was afforded to demonstrate, rather briefly but conclusively, clinical tests to show the direct relationship between the size of the dispersed phase particles and the palatability of the finished emulsion.

The three products "A" (Homogenized), "B" (Mortar), and "D" (Mix-Master) were administered to individuals with the following results:

Case No. 1. Adult (Male). Age 45 years. Occupation: Doctor of Medicine.

SPECIMEN "A" (HOMOGENIZED).

Palatability: Excellent.
Regurgitation: None.
Miscibility: All traces of emulsion removed from the mouth by drinking water.

SPECIMEN "B" AND "D" (MORTAR AND MIX-MASTER).

Palatability: Taste of oil.
Regurgitation: From 4–6 hours after administration.
Miscibility: Water failed to remove all traces of emulsion from the mouth.

Case No. 2. Child (Female). Age 12 years.

SPECIMEN "A" (HOMOGENIZED).

Palatability: Excellent.
Regurgitation: None.
Miscibility: All traces of emulsion removed from the mouth by drinking water.

SPECIMEN "B" AND "D" (MORTAR AND MIX-MASTER).

Palatability: Claimed taste of oil and nausea. Refused further administration.

Case No. 3. Child (Male). Age 9 years.

SPECIMEN "A" (HOMOGENIZED).

Palatability: Excellent.
Regurgitation: None.
Miscibility: All traces of emulsion removed from the mouth by drinking water.

SPECIMEN "B" AND "D" (MORTAR AND MIX-MASTER).

Palatability: Claimed taste of oil and nausea. Refused further administration.

SUMMARY.

In comparing the time of manufacture and the stability of the finished product the Homogenizer produced the best emulsion, requiring only eight (8) minutes. The Mix-Master required but eight (8) minutes for manufacture, producing a satisfactory emulsion for immediate use, but showed stratification in one (1) week. The Mortar emulsion required sixteen (16) minutes for manufacture and produced a satisfactory product for immediate use, but showed stratification after one (1) week storage. The Milk-Shake Mixer proved unsatisfactory in that no emulsion was formed after sixteen (16) minutes of agitation.

CONCLUSION.

The practice of using the ever-ready and handy Milk-Shake Mixer for the manufacture of extemporaneous emulsions is emphatically condemned.

For the manufacture of a satisfactory emulsion the following methods are recommended in the order based upon their palatability and stability. (1) Homogenized. (2) Mix-Master Mixed. (3) Mortar and Pestle.

Further study is needed in order to establish the full value of the Homogenizer to the modern practice of Pharmacy.

PHYSICIANS, PATIENTS AND PRESCRIPTIONS.*

BY CHESTER I. ULMER,[1] M.D.

For several years I worked in the L. L. Walton Pharmacy, Williamsport, Penna. I give you this rather drab bit out of my autobiography, not that it particularly interests you nor to give any unsolicited publicity to the Walton Pharmacy, but rather because it may serve as a protective buffer for some of the remarks I shall now make. Any criticisms I may make of either profession are purely constructive ones and are made with no belligerent spirit, I assure you.

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* Presented before Section on Practical Pharmacy and Dispensing, A. PH. A., New York meeting, 1937.

[1] Physician, Gibbstown, N. J.

Yes, I have worked behind the drug counter. I've had customers hand me their prescriptions and now, years later, I write prescriptions—some of them the very same ones. It was always interesting and sometimes amusing for me to observe the attitude of some people when they brought their prescriptions to our store. Some would triumphantly surrender the prescriptions, revealing in every gesture the fullest confidence in their doctor. Others, apparently somewhat doubtful about its value and probably lacking full confidence in the physician, would slowly come up to the counter, make furtive glances around the store and then would say, "What's in this prescription?" Or, "What's this prescription good for anyway?" Now that's a tough question for any druggist, how should he know when even the doctor himself doesn't know sometimes. Not getting much satisfaction from the first two questions the customer leans confidentially over the counter and says, "What do you think of this doctor anyway—is he really any good?" Well, the druggist doesn't know what to say to that one, and if he does, thank heavens, he just doesn't say it.

Yes, patients study and analyze their prescriptions and it is surprisingly true that many are able to read them. The quantity symbols often perplex them, however. It is a very common practice and most druggists know it—for patients to come into the store, pull out a prescription and often ask for it by name, particularly if it is a one-item prescription. Physicians should always avoid writing prescriptions for an individual item when it can be prevented. I have often seen people come into drug stores with the individual item cut from the prescription and ask the price of it. Why, even certain medical proprietaries of the air capitalize on the importance of having several items on the prescription. What did I hear over the radio the other night, "When your doctor writes a prescription he does not write for just one item, he writes for several!"

Let us discuss briefly the physician and his prescriptions. I am not in accord with that cynic who said that a physician is a man who writes prescriptions till the patient either dies or is cured by nature. For quite a long time it has been observed that many physicians have become indifferent about prescription writing and have developed a tendency to prescribe certain pharmaceutical and proprietary products in preference to official preparations. There are probably two reasons for this negligent prescription attitude of many physicians: *First*, the curricula of many medical schools give students only two per cent of pharmacology and prescription writing, yet the responsibility of that item of medicine to the laity is about twenty per cent. Therefore, a vacuum of eighteen per cent exists and it is within this eighteen per cent that clever systems of advertising prey upon the public, exploiting medicine or pharmacy with little or no consideration of either. After all the physician's *greatest* and most *effective* weapon against disease is a broad knowledge of therapeutics. The second reason, probably, for the growing tendency of physicians to prescribe certain pharmaceutical and proprietary products is because of an indifferent attitude. Many of us have become somewhat indolent and have found it easier to write for a single proprietary item than to look into our Formulary. A physician is often criticized by his more intelligent patient for using a single proprietary item instead of devising an individualized treatment.

There is no doubt that many physicians have gradually allowed their offices to become annexed to the advertising offices of many pharmaceutical manufacturers.

Has American pharmacy retrograded? Many times the pharmacist has to take the barbed criticism from the public that he has allowed his drug store to degenerate into an emporium or lunch retreat. If there has been some deterioration in American pharmacy then the *doctor* must share the responsibility with the druggist for having allowed the prescription department to become subordinated into a mere side issue of a drug store.

There is no doubt that the occasional untactful attitude of some pharmacists has created a feeling of apprehension as to the merit of a prescription when it is presented by the patient. Quite often the druggist sort of shrugs his shoulders when questioned about the efficiency of a prescription when instead he could just as easily inspire confidence.

We have queer companions in both of our professions! We should look at ourselves occasionally, as others see us. There are some bizarre fellows in pharmacy, believe me, and we know it, too. Look at some of our drug stores on Main Street, cluttered up with all kinds of merchandise, counters burdened with a conglomeration of gadgets. I predict that eventually the pendulum is going to swing back, out of all this chaos, back to the more dignified pharmacy of several decades ago.

In medicine we have some weird inhabitants and we know it, too. We have springing up a new cult within our own profession—they are the therapeutic nihilists—those physicians with a smug attitude, who shrug their shoulders at all drug therapy. These iconoclasts have a limited drug armamentarium to say the least, they practice medicine with only three prescriptions, water, fresh air and sunshine. The profession has entirely too many of this type of physician who think that to cure all the ills of all people is to simply write:

℞ Aqua, cong i
Sig: Glassful every hour

(Signed) Dr. Sponge

I must say a few words about the problem of counter-prescribing by pharmacists and medicine dispensing by physicians. Both practices started innocently enough many years ago but have been accelerated during the past six or seven years because of the depressed economic status of the people. Every pharmacist has, at some time, done counter-prescribing and every physician has done some drug dispensing. I have done both.

The fact remains, however, that the physician is no more qualified to engage in intelligent dispensing than the pharmacist is in counter-prescribing. In each case there is the assumption of responsibilities for which neither is fitted. Let each one stick to his trade. Let the pharmacist practice Pharmacy and let the physician practice Medicine. By doing this, confidence and respect will be established on both sides. It will create a better spirit and teamwork between pharmacists and physicians.

To help correct the tendency of proprietary prescribing and to assist in reviving the art of prescription writing a committee from the State Medical Society of New Jersey is coöperating with a committee from the New Jersey Pharmaceutical Association striving for improvement in the writing of prescriptions along ethical lines. This joint committee is known as the Joint Committee on Professional Relations.

Our endeavor is to give to the physicians practical prescriptions which will take the place of certain proprietary preparations. We differentiate between so-called *proprietary preparations* and *ethical specialties*.

A little more than a year ago, in April 1936, we commenced the simultaneous publication in both of our Journals—the JOURNAL OF THE PHARMACEUTICAL ASSOCIATION and the *Journal of the Medical Society*—a group of six seasonable, ethical formulas. They appeared bimonthly. These prescriptions have proved very popular and practical with many of the physicians in our state and I am sure that many New Jersey pharmacists have already noted that doctors are prescribing more of these ethical formulas.

It is gratifying to note that in one of the hospitals of our State of New Jersey our prescriptions are being used as a basis for instruction at the weekly conferences with the interns and medical staff.

In addition to our prescription feature we plan to present in the JOURNAL OF THE PHARMACEUTICAL ASSOCIATION and the *Journal of the State Medical Society* a series of articles covering recent developments in the use of therapeutic agents and new drugs. In the August issue of our respective Journals appeared a timely article sponsored by our Joint Committee on Professional Relations on Sulfanilamide.

In New Jersey we are going on with our work. It has really just begun.

PHARMACY AND MEDICINE—two great allied professions. With so many common interests it is most important that we face together the inevitable problems of the future.

IS THE PHARMACIST A POOR MERCHANT BECAUSE HE LACKS TRAINING IN ACCOUNTING, ECONOMICS AND BUSINESS STUDIES?*

BY RALPH R. KREUER.[1]

I have asked a question as the title of my paper, and, while I admit that it is difficult for anyone to answer that question, I am convinced, after much study and personal observations, that the answer is, "yes." Yes, the pharmacist is a poor merchant because he lacks these necessary and vital requirements.

I have seen conditions in drug stores that were appalling. I have seen pharmacists going about their daily duties with absolutely no idea of how much business they must do in order to make a profit. They could not tell whether they were heading for bankruptcy or actually making money. They could not discuss a profit-and-loss statement or stock control. Many pharmacists haven't the least idea of what their inventory is, for the simple reason that they don't take one. I have asked many pharmacists if they were making a profit on their soda fountains. The answer is usually, "yes," or "I believe so." On further questioning as to how much money they were making, or what gross profit they realized, the answer was almost invariably "I don't know." Keen competition usually takes its toll of these poor business men, because they lack the weapons with which to fight their com-

* Presented before the Section on Practical Pharmacy and Dispensing, A. PH. A., New York meeting, 1937.

[1] Professor, Duquesne University, School of Pharmacy.

petitors. In times of close competition a merchant must call upon his reserve training and experience in waging his battle. When business comes easy, some merchants are satisfied with their sales and are inclined to allow their commercial knowledge to lie dormant, then when an emergency arises or an economic crisis takes place, the methods of increasing sales that he once knew have become obsolete or ineffective. In other cases where the merchant has never had training in business methods, he is almost at a total loss how to meet competition.

A pharmacist, like any other merchant, should have his finger on the pulse of his business at all times, so that he may immediately detect any change in his organization. In order to do this he must constantly know its condition, just as a physician watches for a change in his patient. If a change for the worse takes place, the doctor applies the proper stimulant. But if the pharmacist is ignorant of his business ailment, he doesn't know what stimulant to use or where to apply it.

Every one is familiar with the survey made a few years ago by the U. S. Department of Commerce, in which a number of failed drug stores were studied in order to determine the causes of failure. Some very interesting results were obtained from this survey, but the one outstanding cause of failure which impressed me was poor business management. The creditors stated that 73% of the stores failed because of incompetency or inability to manage the stores. This covers a large variety of offences, but I believe it can be boiled down to the statement that the owners lacked the knowledge of, what I shall call, business principles, disregarding the fact that some pharmacists are merely lazy. Too many pharmacists have little or no knowledge of the essentials necessary to conduct a profitable business. Too many of them are content to sit back and bemoan the fact that their competitors are ruining their trade. Competition is very often used as an excuse to cover up personal shortcomings.

In the survey I have mentioned, competition did not seem to be a direct or important cause of failure in any of the stores. I have referred to this survey merely to supplement my own observations in the thirty-odd stores in which I have worked.

Where, then, does the fault lie? Is it because the owners and managers were never taught these important subjects of accounting, economics and various other business studies? Or is it the fault of the pharmacy schools? I have examined the pharmacy bulletins of practically every school of pharmacy in the country, and I find that every one offers some type of business course as a part of its curricula. In some schools these courses are optional. Still, the fault may be because in some schools of pharmacy the courses in economics, etc., are not taught by the faculty of the pharmacy school, but by the regular department of commerce, by men who are not pharmacists and who are not familiar with the pharmacists' problems. The fact remains, however, that to-day pharmacy schools are offering these courses. Changes have been suggested, and some will undoubtedly take place. It has been proposed that a combination course in pharmacy and business, consisting of five years, be inaugurated. It is unfortunate that the older pharmacists did not have the advantages of courses in business training and economics when they attended college. This does not mean, however, that the man already in business, and who is without this vital training, is lost. He is not lost if he has the initiative and the courage to acquire this knowledge for himself. How? There are several ways. One is by attending classes in business colleges and universities in his spare

time, and the other is by reading texts that deal with these subjects. Our drug journals carry very excellent articles on business management, salesmanship, accounting, etc., and these are certainly within reach of every pharmacist.

ADVERTISING PROFESSIONAL PHARMACY.*

BY LIEUTENANT JOSEPH A. ORTOLAN.[1,2]

Economic conditions have arisen in pharmacy during the past twenty-five years making it very difficult for the average pharmacist to eke out a living in keeping with his education, training and responsibility.

In a majority of instances these economic conditions have been brought about not by any one particular cause but a number of different reasons: Advancement in Medicine, a greater number of students taking up the profession of Pharmacy, depressions and unemployment.

Slowly, but surely, the pharmacist has had to increase the already large and overwhelming number of items in his pharmacy in an effort to meet competition with the merchants in his locality. These items, most of them entirely foreign to a professional pharmacy, were a means toward an end. These goods and the luncheonette were used as a method to have people come to his pharmacy (which was in reality more of a store than a pharmacy). So-called customers came only in an emergency, usually when the merchants really having a right to sell these goods had their places closed because of the late hour, Sundays and holidays. Then the customer had no alternative except to go to the neighborhood pharmacy and reluctantly pay the price charged for the item.

In this the average pharmacist believed he was making a profit, whereas in reality he was making a scanty living, if he was really making a living at all. His success, if any, was problematical. However, this is just so much water over the dam.

The thing that annoys the professional, thinking and intelligent pharmacist to-day is that, granted for the sake of argument that he ekes out an existence, he has had to pocket his pride, lose his prestige and finally lose the professional attitude which is his rightful heritage and which heritage belongs to all thinking, deserving and intelligent pharmacists who do their work well and conscientiously. These men are proud of the fact that they belong to an ancient and honorable profession allied with medicine and dentistry.

The dawn of a new day is coming for the pharmacist, however. To my way of thinking, the pharmacy of to-morrow will not be located, as it is in many instances at present, on the corner of a building occupying perhaps the most expensive piece of real estate on the block.

Rather I want you thinking pharmacists, both men and women, to visualize, if you will, the ideal as well as the practical professional pharmacy of the future, situated either on the first, second, third and even fourth floor of an up-to-date building. Here we would have better light and ventilation, even though we have

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* Presented before the Section on Commercial Interests, A. Ph. A., New York meeting, 1937.

[1] Medical Corps, U. S. Navy, retired.

[2] Instructor, Brooklyn College of Pharmacy.

air conditioning of buildings. The rent would be lower, the overhead less, and what is more important, there would be a professional atmosphere not ordinarily found in the corner pharmacy.

We are creatures of custom. It has been brought to my attention that a few teachers of economics in schools of pharmacy try to instil in the minds of the embryonic pharmacists that the corner is the logical and most profitable location for a pharmacy. However, if pharmacy wishes to emerge from the doldrums and lethargy it has been placed in, it must rise (figuratively and literally) above its present location or level.

Why work for the landlord? Why pay him several hundreds of dollars a month for the privilege of having your pharmacy on the corner, next to and on the same level as the butcher shop, grocery store, saloon, millinery store or some other shop? Haven't you any professional standing or are you just a shop-keeper like the butcher or grocer, who has never seen the inside of a college? My dear pharmacists, haven't you any respect for yourselves to place yourselves on the same plane as the storekeeper?

There is no more reason why the professional pharmacy should be on the ground floor, at or near the corner with its expensive and almost prohibitive rent, than it is for a physician, dentist and/or attorney to have his office on the corner.

Bear in mind that the pharmacist of to-day is supposed to be an intelligent individual, well learned and well educated along academic and professional lines.

If this is true, and we have every reason to believe it is, then the pharmacist must create a favorable, professional atmosphere and conduct himself along professional lines and abandon those old, worn-out fallacies given him by well-meaning but ill-advised individuals lacking foresight.

You may ask how will physicians and the public (especially the latter) know where I am located? By ethical advertising in the same professional way that physicians, dentists and lawyers make known their location. By the simple and expedient way of mailing neat, professional cards to families and individuals in the locality where you are going to establish your pharmacy.

Why in heaven's name practice pharmacy if, after having spent your time and money studying this art, you open up a lunch room or a vest pocket edition of a general store as many pharmacists have done in the past and are doing to-day? If you had this in mind it would have been far more advantageous if you had majored in merchandising in a university and come out as a merchant.

Pharmacy must divorce itself of those items not properly belonging to an ethical and professional pharmacy. When this is accomplished by concentrated action on the part of the pharmacists all over the country, the medical and dental professions as well as the public will then recognize and acknowledge pharmacy as a profession and place the pharmacist on a professional standing where he rightfully belongs.

You may make the statement that physicians and dentists sell services. So does a pharmacist. If you do not believe a pharmacist sells his services then you have failed to learn the first elementary principles of economics.

Instead of cluttering up his place with non-professional items, costly, and with a tremendous outlay of capital, including a vast space allotted to a luncheonette, let the pharmacist learn the selling of hospital equipment and appliances, the

fitting of trusses, abdominal belts, elastic hosiery and such other items as are required in the sick room and hospital and then equip his pharmacy with these items. In addition let him enlarge his present stock of biologicals, such as vaccines, serums, antitoxins, et cetera. Let the pharmacist impress the physicians of his ample stock of these biologicals. These items belong in a well-equipped and properly conducted professional pharmacy—not alarm clocks, books, magazines, candies, tobaccos, cigarettes and cosmetics.

Imagine if you will, please, an unfortunate individual who has just come from his physician to your pharmacy to have his prescription compounded. He enters your so-called modern pharmacy (the only thing the pharmacist has that is modern is the radio and mechanical refrigerator) and he is lost in the maze of bewildering items foreign to an ethical pharmacy. The patient, though ambulant, has to be piloted as it were to the prescription department. While waiting for the pharmacist to compound his prescription, his olfactory nerves pick up the odors of fried bacon and eggs or hamburger and onions. Cooking odors at best are far from pleasant, and to the majority of patients repulsive and nauseating.

The patient may begin to wonder if he is in a pharmacy or if he has made a mistake and entered a lunch wagon. Do you wonder, brother pharmacists, why the gag man on the vaudeville stage or on the radio uses that old joke, insulting to the intelligence of all thinking pharmacists, that in order to graduate from a college of pharmacy, the candidate must be able to make sandwiches? I do not wonder, rather my blood boils when I hear that gag.

If our great and illustrious pharmacists such as Louis Pasteur, and a great many others, could view the average pharmacy of to-day they would in all probability turn over in their graves and wish to remain there.

A word or two about cleanliness is not out of place. It plays a very important part in advertising your pharmacy. Too many pharmacists, I regret to state, fail to keep their pharmacies spotless. I mention this because I have had many physicians, dentists and laymen ask me if we as teachers in a school of pharmacy teach students cleanliness. The answer is obvious. My answer to you, however, is that some pharmacists are indolent. Soap and water are inexpensive.

When you are sure your place is clean and well organized, invite all of your physicians, dentists and a number of your prominent and influential friends in your neighborhood to visit and inspect your pharmacy. Show them about. Point out different features about your place. These people in turn will tell others about your establishment and in this way advertise it as a pharmacy and not a drug store. This latter term is hackneyed, obsolete and a misnomer. Let us all try to educate the public to call it a pharmacy and not a drug store. Another term which is a misnomer and should be relegated to the sea of oblivion is the term clerk. Let us use the more dignified name of John Doe's assistant; not John Doe's clerk.

The professional pharmacist should be a missionary. He should call on his physicians, dentists, lawyers and church people in the neighborhood. Learn to cultivate their friendship. Let them know you are awake and alive.

Keep your physicians and dentists informed about Pharmacopœial and National Formulary preparations. Suggest to them the new and unofficial remedies. Realize that the busy practitioner has no time to delve into pharmaceutical preparations. Take time out to explain concisely and intelligently any U. S. P. and N. F.

product the physician and dentist may appear interested in or you may wish to interest them in or desire information about. But please, Mr. Pharmacist, be well informed yourself so that you may be able to answer intelligently any questions he may want to ask about a product. In this, as in all matters between physician and pharmacist, be tactful and diplomatic.

Colleges of pharmacy in general, and teachers of economics in particular, will find this topic a never-ending source of interest among students of pharmacy, owners of pharmacies and their assistants.

The colleges are doing their part in elevating pharmacy. It is up to the pharmaceutical organizations and their members to bend all their energies in creating among the medical, dental and legal professions, as well as the public, the professional aspect pharmacy deserves and have them recognize the responsibility placed upon the pharmacist in the community, from a professional angle.

NO ONE WISHES TO SEE A MORGUE.*

BY CHARLES E. ELY.[1]

Most of the side windows of independent drug stores can be made interesting and attractive to the communities which they serve, with a little thought and effort. For instance, in a ten-foot window, construct a bill-board of celotex, four by eight feet, with a post at each end, a green lattice at the bottom, a green grass mat cut lengthwise, and if available an artificial shrub or evergreen in front of each post to complete the out-door effect. Effective bill-board lighting should be used and an azure-blue curtain at the back will help. This background arrangement can be changed from time to time.

A weekly display of material should be planned in advance and advantage should be taken of each holiday, particularly New Year's Day, Lincoln's Birthday, Washington's Birthday, Easter, Independence Day, St. Valentine's Day, Armistice Day, Thanksgiving and Christmas. A Fourth of July display, for instance, might have a copy of the Declaration of Independence prominently placed on the bill-board, a stock of picnic supplies arranged on the grass at one side and an American flag at the other side, with a small electric fan at the rear so placed as to keep the flag in motion. Appropriate show-cards will link the merchandise with the holiday and emphasize the importance of its celebration.

The opening of school, safe-driving campaigns, the prevalence of certain diseases, the importance of regular examinations by the physician and the dentist, the observance of public health measures, historical facts about the community, cartoon and other contests by children, prescription and other professional services offered by the drug store, Pharmacy and First Aid Weeks, the work of church, hospital, charitable and civic organizations and local matters and local points of interest can be made the theme of weekly displays.

Special merchandise can also be used for such displays. Each piece of stationery from a box may be pinned at random over the bill-board, the empty box

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* Presented before Commercial Interests Section, A. PH. A., New York meeting, 1937.

[1] Professor, Detroit Institute of Technology.

placed at the bottom with a sign reading "Look! You get all of this fine stationery for 29¢." A soap display with a suitable card on the bill-board reading "5¢ a bar," and five bars of the soap each placed on a doily elevated a few inches from the floor, as if they were gems, will make an effective appeal. Boy Scout and First Aid kits, greeting and birthday cards, bridge prizes, rubber goods, sick-room supplies, vacation articles, prescription files and apparatus, special drugs and preparations and many other similar articles make attractive displays.

An invitation to the public to offer suggestions for displays for the window creates interest and brings out good ideas, and occasional invitations to physicians, dentists, nurses, public officials and groups of patrons to inspect certain displays will help build up interest in your pharmacy.

With some thought and effort and expense, that side window will become a hobby and a helper, and can be made to influence many in your community as well as transients every week.

Remember, that while the roof of your building is the sky-line, the show window is the eye-line. It pays to use 100% of your eye-line!

RESEARCH—DR. CARREL.

According to the *New York Times Magazine*, Dr. Alexis Carrel has passed his sixty-fifth year, and in keeping with the ruling of the Rockefeller Institute should retire this year; but despite this rule he will continue another year. In recent work he has coöperated with Col. Charles A. Lindbergh, and after Dr. Carrel's retirement he will carry on his investigations in his private laboratory.

FUNDS OF THE COMMONWEALTH TO BE USED TO AID GRADUATE EDUCATION.

Medical News reports that a grant to be disbursed over a period of five years has been given to the University of Minnesota by the Commonwealth Fund, New York, to be used for medical education. The fund will provide a series of advanced courses in at least five branches of Medicine.—*Jour. A. M. A.*

PRIZES FOR THIRD- AND FOURTH-YEAR HIGH SCHOOL STUDENTS.

Incentives are offered by the American Association for the Advancement of Science, acting in collaboration with the Cinchona Products Institution of New York and Amsterdam, in the nature of an essay on "Quinine in History," to be participated in largely by High School students of the third and fourth years, of the Southern and Southwestern states.

One of the unequivocal blessings of a really good education is that it spurs the mind on to a sublime and enduring discontent with any particular stage of achievement and inspires men, contemptuous of the advancing years, to strive for ever wider vision, for continued victories over new territory.—JAMES ROWLAND ANGELL.

MINNESOTA STATE BOARD OF PHARMACY.

The Minnesota State Board of Pharmacy gave an examination for registration as pharmacist the week of July 11th. Forty-four were successful in passing this examination. A large majority of those passing were graduates of the University of Minnesota College of Pharmacy. Every one of the graduates of the Minnesota University was successful in passing the examination, and remarks were made by one of the older members of the Board relative to the excellency of the papers of these young pharmaceutical graduates.

The Board of Pharmacy also passed additional regulatory measures in an endeavor to make the Pharmacy Law a better public health measure.

REGULATION NO. 5.

Whenever a pharmacy or drug store changes its location, it shall apply to the Board of Pharmacy for amendment of its annual permit to cover the new location. There shall be no charge for such amendment.

REGULATION NO. 6.

No permit shall be issued for a pharmacy or drug store which is kept open more than fifty-six hours per week, unless at least two registered pharmacists are employed in such pharmacy or drug store on a schedule that will assure the presence of one registered pharmacist at all times. This regulation shall not apply where the owner of a pharmacy or drug store is a registered pharmacist and is continuously and personally in charge of such pharmacy or drug store.

The Board took up the matter of classifying poisons into groups together with official antidotes, which is in line with the progressive pharmaceutical policies of other states such as California, New Jersey, Colorado and others. Other important regulatory measures were taken up for further study and consideration.

Plans were discussed for the meeting of the National Association of Boards of Pharmacy to be held in conjunction with the AMERICAN PHARMACEUTICAL ASSOCIATION and the American Association of Colleges of Pharmacy, August 21st–26th. The meeting of the Pharmaceutical Law Enforcement group with such men as Dr. Robert L. Swain of Maryland; Dr. Robert P. Fischelis of New Jersey; P. H. Costello of North Dakota; and S. H. Dretzka of Wisconsin, promises to be a most interesting one. Dr. Swain, chairman of this group, hopes to have a model pharmacy law well developed by the time the meeting rolls around. His discussions along this line will be of interest to every pharmacist in America. Every pharmacist in the Northwest should avail himself of this remarkable opportunity to hear the leaders in the pharmaceutical world who will be present at the A. PH. A. meeting.

ALLOTMENTS TO THE STATES UNDER THE LAFOLLETTE-BULWINKLE ACT.

The U. S. Public Health Service has announced the following allotments, amounting to $2,400,000, from the first year's appropriation of $3,000,000. The states will be expected to supplement these allotments by appropriations from their own funds.

Alabama, $72,294; Alaska, $2701; Arizona, $9639; Arkansas, $47,650; California, $103,791; Colorado, $18,228; Connecticut, $26,518; Delaware, $5283; District of Columbia, $16,860; Florida, $38,055; Georgia, $77,134; Hawaii, $8088; Idaho, $8019; Illinois, $125,299; Indiana, $59,380; Iowa, $43,564; Kansas, $31,909; Kentucky, $57,318; Louisiana, $50,871; Maine, $13,486; Maryland, $31,892; Massachusetts, $69,623; Michigan, $77,206; Minnesota, $42,324; Mississippi, $54,119; Missouri, $67,376; Montana, $8575; Nebraska, $22,833; Nevada, $2475; New Hampshire, $8775; New Jersey, $71,327; New Mexico, $9023; New York, $193,724; North Carolina, $84,259; North Dakota, $12,340; Ohio, $110,784; Oklahoma, $46,342; Oregon, $16,077; Pennsylvania, $165,082; Rhode Island, $12,062; South Carolina, $52,522; South Dakota, $12,420; Tennessee, $66,644; Texas, $128,950; Utah, $9183; Vermont, $6286; Virginia, $58,983; Washington, $25,358; West Virginia, $32,297; Wisconsin, $45,368; Wyoming, $4148; Porto Rico, $34,587; and Virgin Islands, $949.00.

A considerable portion of these large sums will be expended for the purchase of drugs, medicines and medical supplies, under the direction of the state health authorities, for the venereal disease campaign.

PERSONAL AND NEWS ITEMS.

CORRECTION.

In the JOURNAL, Vol. 27, No. 6, June 1938, "Toxicity of Propylene Glycol, by J. H. Weatherby, Ph.D., and H. B. Haag, M.D., page 468, Table II, average Subcutaneous Lethal Dose 5.6 (3) for the mouse should be in column headed Diethylene Gm./Kg., instead of in column headed Ethylene Gm./Kg.; page 470, Table III, the next to the last line should read: "Saline 0 0 0 0 0 x xxxx xxxx xxxx xxxx," rather than as it appeared in the manuscript.

Rhode Island Pharmaceutical Association, which met recently, enjoyed a most interesting convention in spite of continued rain. Both the business and entertainment features got a big hand. Among the recommendations in the President's address was one to send annually to Frank H. Jackson, the only living charter member of the R. I. Ph. A., a floral tribute. Governor R. E. Quinn pledged the coöperation of his administration with the state's pharmacists.

OHIO MEDICAL MEN SEEK RESTRICTIONS.

A resolution asking a state law forbidding the sale of hypnotic drugs and sedatives without a prescription, and asking the Ohio Medical Association to initiate a movement toward this end, was passed here recently at a meeting of the Cincinnati Academy of Medicine.

VENEREAL FUNDS WON'T HELP OHIO Rx MEN.

Not one dollar of the $110,784 allotted for prevention, treatment and control of venereal diseases under the LaFollette-Bulwinkle Bill will be spent for purchases from retail pharmacies, Dr. Walter Hartung, Ohio Director of Health, declared last week. Such a practice would be contrary to the state's purchasing system, he stated.

The twenty-first annual Chemists' Convention met in Ottawa, Canada, June 20th to 22nd. *Industrial and Engineering Chemistry* for July 20th carries a large number of illustrations of the Convention. More than six hundred registered, among them eighty-five British and fifty American chemists.

The Mississippi Pharmaceutical Association, which met in Gulfport, was a victory celebration for Fair Trade. This was one of the most successful and constructive conventions held in the state. Attendance was good and the program very interesting.

Montana Pharmaceutical Association met at Great Falls, July 15th and 16th. A most successful convention was held; attendance was good, business sessions interesting and social features enjoyable.

North Dakota Pharmaceutical Association met in Mandan, where one of the most important conventions of the Association was held. The speaking program was well-rounded and the entertainment provided, excellent.

South Carolina Pharmaceutical Association met in Columbia recently. Fair Trade received its share of attention. Among the speakers were: Dr. Lew Wallace, Laurel, Miss.; R. C. Stokes, of the Medical College; and W. D. Strother, University of South Carolina. An Open Forum on professional problems was conducted. Attendance was large and the meeting successful.

Maine Pharmaceutical Association met in Poland Springs, when Maine's druggist-governor, Lewis O. Barrows, of Newport, was honored. An ovation of several minutes was extended upon the Governor's arrival. On the opening day of the convention Governor Barrows was renominated by an overwhelming majority.

Virginia Pharmaceutical Association met in Roanoke, recently. Fair Trade in Virginia was pronounced a definite success. Socialized Medicine came up for discussion, and many interesting addresses were made.

Washington State Pharmaceutical Association was host to Oregon State Association on July 11th to 13th, returning the compliment to Oregon Association, which was host to Washington and Idaho Associations at Portland in 1935. Although Idaho Association had met, many of its members attended the sessions in Seattle.

The Nebraska Pharmaceutical Association has issued a publication called "The Nebraska Mortar and Pestle," the first issue having appeared in June. Congratulations!

Judge George C. Blakeley, The Dalles, Oregon, celebrated his 52nd year of business in the same store, recently. Many congratulations were received by him in behalf of his remarkable business record, as well as his active participation in the civic life of his town. The Blakeley family is noted for its longevity, his father having passed the hundred mark.

Mr. Blakeley is a past-president of the Oregon State Pharmaceutical Association, a Life Member of the A. PH. A., and has also served on the Oregon Board of Pharmacy.

One of The Dalles papers paid the following tribute: "Friends who have followed his career in The Dalles during the half century know he has carried out the best traditions of his heritage with an unfailing courtesy and kindliness in his personal contacts, with a ready interest in every deserving cause, and with a tolerant attitude toward his fellows which has made friends of all and enemies of none."

Dr. John C. Krantz, Jr., Professor of Pharmacology, School of Medicine, University of Maryland, Baltimore, will enjoy a six weeks' tour of England, France, Switzerland and other European countries, and while abroad will present two papers before the International Physiological Congress in Zurich.

The Pharmaceutical Journal of Great Britain, July 2nd, devoted considerable space to the Food and Drug Law recently passed. Among statements made is the one that this Bill marks a great advance over existing law and clears up a great many points which were not perfectly clear in the old law.

The North Carolina Pharmaceutical Association met in beautiful Asheville, recently. A business and entertainment program, unequaled in the history of the Association, was carried out. The attendance was large, and the addresses were splendid. Among the speakers were: Dr. Carl V. Reynolds, State Health Officer; Dr. Neal Bowman, Temple University, Philadelphia; and Mr. Rowland Jones, Jr., Washington representative of the N. A. R. D. Among other things discussed was Fair Trade.

The Alabama Pharmaceutical Association met in Birmingham this year. A new and interesting event was the Past-Presidents' Dinner, with members of the State Board of Pharmacy as hosts. The convention was well attended and a number of interesting speakers were enjoyed.

The Northern Ohio Druggists' Association has announced a prize contest for the best papers on "How You Would Operate a Drug Store," among senior students of Western Reserve University School of Pharmacy. The first prize of $25.00, and smaller prizes, including $1.00 to all submitting papers according to the rules, will be awarded at the regular meeting of the Association, October 7, 1938.

$250,000 has been allocated for the construction of a Pharmacy-Chemistry Building at the Montana State University.

The 53rd annual meeting of the Tennessee Pharmaceutical Association was held in Knoxville, July 18th to 20th. Dr. W. P. Winters of Nashville presided and delivered an address. One of the highlights of the convention was the Secretary's Report on the Tennessee Fair Trade Bureau. Among the speakers were: J. W. Snowden, Aurora, Ill., "Prescriptions Need Marketing;" Rowland Jones, Jr., Washington representative of N. A. R. D., "The Washington Picture;" Dr. Herbert Acuff, Knoxville, "The Interrelationship of Medicine and Pharmacy;" and Dr. Ralph W. Clark, Rahway, N. J., "Increasing the Prescription Business."

The Arkansas Pharmaceutical Association met in Hot Springs recently, and a most satisfactory meeting was enjoyed. Col. T. H. Barton spoke on "Opportunities of Arkansas" and Ralph A. Beegle on, "The Commercial Side of Pharmacy," which talk had a wealth of information for the progressive druggist.

The Massachusetts Pharmaceutical Association met at Swampscott recently. A large attendance was recorded, and all reports showed the Association to be in good condition. Reports of the Fair Trade Commission and of the Legislative Committee were outstanding in their interest to the convention.

A fine attendance of druggists took part in the 48th convention of the Colorado Pharmaceutical Association at Glenwood Springs. An outstanding program and delightful surroundings drew a record turnout. Fair Trade was one of the featured discussions and altogether a very successful meeting was held.

Ohio State Pharmaceutical Association met in Dayton, July 25th to 28th. An interesting feature of the meeting was a Reunion and Dinner for Pioneer Pharmacists of Ohio